LXXXV. STUDIES IN THE BIOCHEMISTRY OF MICRO-ORGANISMS.

XXX. THE MOLECULAR CONSTITUTION OF THE METABOLIC PRODUCTS OF *PENICILLIUM BREVI-COMPACTUM* DIERCKX AND RELATED SPECIES.

I. THE ACIDS $C_{10}H_{10}O_5$, $C_{10}H_{10}O_6$ AND $C_{10}H_{10}O_7$.

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In Part xxiv of this series [Clutterbuck, Oxford, Raistrick and Smith, 1932] it was shown that out of 15 species in the *P. brevi-compactum* series submitted to examination, 14 metabolised glucose into a mixture of phenolic acids, *viz.* mycophenolic acid $C_{17}H_{20}O_6$ (formed by 12 species only), and the new metabolic products $C_8H_6O_6$, $C_{10}H_{10}O_5$, $C_{10}H_{10}O_6$ and $C_{10}H_{10}O_7$, the last two being detected in every instance and the first two being probably formed by all 14 species although not always detectable with certainty on account of the smallness of the respective yields. The question of the molecular constitution of mycophenolic acid is dealt with in the accompanying paper [Clutterbuck and Raistrick, 1933] and the substance $C_8H_6O_6$ has been shown to be 3:5-dihydroxyphthalic acid [Oxford and Raistrick, 1932]. The present paper deals with the elucidation of the molecular constitutions of the three remaining substances, the acids $C_{10}H_{10}O_5$, $C_{10}H_{10}O_6$ and $C_{10}H_{10}O_7$.

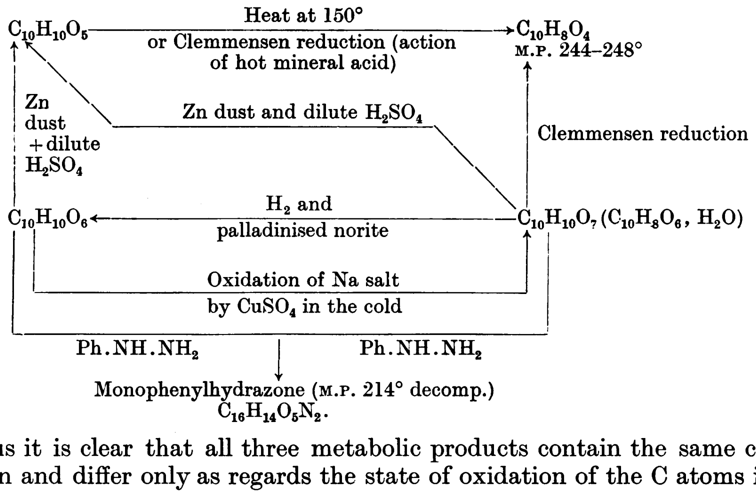
It will be convenient first to summarise the more obvious properties of these compounds as recorded in Part xxiv. All three are optically inactive. All give intense reddish-purple colorations with ferric chloride. $C_{10}H_{10}O_5$ and $C_{10}H_{10}O_6$ (but not $C_{10}H_{10}O_7$) give solutions in cold concentrated H_2SO_4 which fluoresce blue in ultra-violet light, while $C_{10}H_{10}O_6$ and $C_{10}H_{10}O_7$ (but not $C_{10}H_{10}O_5$) give very dark colours when gently warmed with this reagent. All titrate more nearly as dibasic than as monobasic acids, but the end-point is very indefinite since the substances give brownish-red solutions with excess of alkali.

All three melt with elimination of water and some CO_2, $C_{10}H_{10}O_5$ at 145–153° losing nearly one molecule of water (and also 0·1 mol. CO_2) to give as chief product an entirely new substance $C_{10}H_8O_4$; $C_{10}H_{10}O_6$ at about 190–200° with elimination of water to give a crude product not yet examined further, also melting at 190–200° but without effervescence; $C_{10}H_{10}O_7$ at 125–135° losing $1H_2O$ (and practically no CO_2) to yield a syrup which reverts to the original substance when crystallised from solvents containing only traces of water.

$C_{10}H_{10}O_5$ and $C_{10}H_{10}O_7$ are ketones giving immediate yellow precipitates with Brady's 2:4-dinitrophenylhydrazine reagent, while $C_{10}H_{10}O_6$ gives a precipitate with this reagent only on long standing. None of the compounds restores the colour to Schiff's reagent or has any other aldehydic properties.

It was soon established that the loss of water on heating the substance $C_{10}H_{10}O_7$ does not result in any fundamental constitutional change, and hence for all practical purposes the formula may be written $C_{10}H_8O_6, H_2O$. Thus the mono-2:4-dinitrophenylhydrazone (precipitated by the addition of Brady's reagent) when recrystallised and dried at 50° *in vacuo* analysed correctly for the dinitrophenylhydrazone of $C_{10}H_8O_6$. It is significant, however, that this derivative takes up $2H_2O$ when exposed to moist air. More convincing still, the catalytic reduction of $C_{10}H_{10}O_7$ yielded $C_{10}H_{10}O_6$ identical with the metabolic product of this formula, and, since oxygen atoms are not usually eliminated and replaced by hydrogen under the conditions employed, it follows that the original compound must have lost the elements of water under the mild conditions of the reduction.

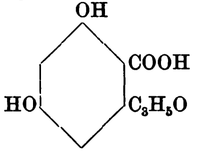
The obvious working hypothesis that all three substances are closely related, naturally led to attempts to effect inter-conversions *in vitro*, and these attempts met with considerable success. For example the sodium salt of $C_{10}H_{10}O_6$ was found to reduce copper sulphate in the cold (although neither $C_{10}H_{10}O_5$ nor $C_{10}H_{10}O_7$ reduces Fehling's solution even on boiling) some $C_{10}H_{10}O_7$ being formed. Further, as mentioned above, the catalytic reduction of $C_{10}H_{10}O_7$ (*i.e.* $C_{10}H_8O_6, H_2O$) yielded $C_{10}H_{10}O_6$, while $C_{10}H_{10}O_7$ and $C_{10}H_{10}O_6$ both gave rise to $C_{10}H_{10}O_5$ when reduced by zinc dust and dilute sulphuric acid at 80–100°. Reduction by Clemmensen's method rather unexpectedly converted both $C_{10}H_{10}O_5$ and $C_{10}H_{10}O_7$ into $C_{10}H_8O_4$, the dehydration product of $C_{10}H_{10}O_5$. Finally it may be mentioned that both $C_{10}H_{10}O_6$ and $C_{10}H_{10}O_7$ ($C_{10}H_8O_6, H_2O$) yield the same monophenylhydrazone $C_{16}H_{14}O_5N_2$. Thus $C_{10}H_{10}O_7$ ($C_{10}H_8O_6, H_2O$) is a ketone and $C_{10}H_{10}O_6$ is oxidisable to the same ketone by phenylhydrazine. These relationships are summarised in the following scheme:



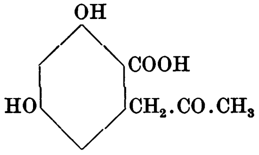
Thus it is clear that all three metabolic products contain the same carbon skeleton and differ only as regards the state of oxidation of the C atoms in the side-chains attached to the benzene ring. Hence, since the oxidation of $C_{10}H_{10}O_7$ ($C_{10}H_8O_6, H_2O$) by alkaline hydrogen peroxide was found to yield some 3:5-dihydroxyphthalic acid, each of the three C_{10} compounds must be a resorcinol derivative with two side-chains *ortho* to each other. This was conclusively proved by the isolation of 3:5-dimethoxyphthalic acid in good yield by the oxidation of the fully methylated derivative of $C_{10}H_8O_4$ by alkaline $KMnO_4$ in the cold.

Since all the oxygen atoms in $C_{10}H_{10}O_5$ can be accounted for (two in —COOH, two as phenolic OH groups and one in a carbonyl group), the possibility of a second hetero-ring attached to the benzene nucleus is ruled out, and the only possibilities for the complete carbon skeleton are $\mathrm{C_6}\!<\!{}^{\mathrm{C}}_{\mathrm{C_3}}$ and $\mathrm{C_6}\!<\!{}^{\mathrm{C_2}}_{\mathrm{C_2}}$. The second possibility can be dismissed at once since there is conclusive evidence that the carboxyl group in $C_{10}H_{10}O_5$ is directly attached to the benzene ring. The dimethyl ether of $C_{10}H_{10}O_5$, *i.e.* $C_{12}H_{14}O_5$, loses both H_2O (0·9 equivalent) and CO_2 (0·1 equivalent) when heated at 180°. The product when hydrolysed by hot alkali yields chiefly the original $C_{12}H_{14}O_5$ together with a relatively small amount of a neutral oily ketone, which can be extracted from the alkaline hydrolysis liquid by ether. This ketone when oxidised by alkaline permanganate in the cold yields not 3:5-dimethoxyphthalic acid but 3:5-dimethoxybenzoic acid identical with an authentic specimen obtained by the oxidation of the dimethyl ether of orcinol.

Hence we may write $C_{10}H_{10}O_5$ as

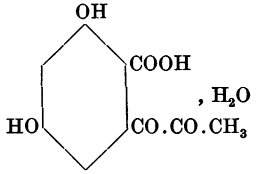


and since it is a ketone the side-chain $-C_3H_5O$ may be either $-CH_2.CO.CH_3$ or $-CO.CH_2.CH_3$. There is little doubt that the former formula is correct since the dimethyl ether of $C_{10}H_{10}O_5$ (like benzylmethylketone, unlike propiophenone) yields iodoform when oxidised by hypoiodite under the conditions prescribed by Birkinshaw and Raistrick [1931] for the estimation of kojic acid. A control experiment with 3:5-dimethoxyphthalic acid showed that the benzene ring in fully methylated resorcinol derivatives is quite stable under these conditions. Hence $C_{10}H_{10}O_5$ must have formula I, and $C_{10}H_{10}O_7$ ($C_{10}H_8O_6$, H_2O) can therefore be represented only by formula II, while $C_{10}H_{10}O_6$ must have formula III or a tautomeric form with side-chain $-CO.CHOH.CH_3$, $-C(OH){=}C(OH)CH_3$, $\underset{\diagdown\mathrm{O}\diagup}{\mathrm{-CH-C}}\mathrm{(OH)CH_3}$ or $\mathrm{-}\underbrace{\mathrm{C(OH)-CH}}_{\mathrm{O}}\mathrm{.CH_3}$, or an equilibrium mixture of two or more of these possible tautomerides.



(3:5-dihydroxy-2-carboxybenzylmethylketone)

I. $C_{10}H_{10}O_5$



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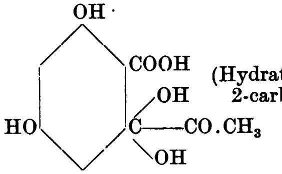
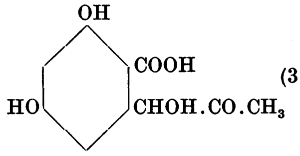


(Hydrated form of 3:5-dihydroxy-2-carboxybenzoylmethylketone)

II. $C_{10}H_{10}O_7$ ($C_{10}H_8O_6$, H_2O)

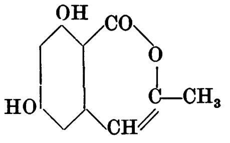


(3:5-dihydroxy-2-carboxyphenylacetylcarbinol)

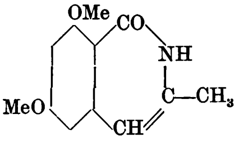
III. $C_{10}H_{10}O_6$

It remains to be shown that these constitutional formulae are in full agreement with all the known properties of the three C_{10} metabolic products. As will be mentioned later there are, however, one or two highly interesting exceptions in the case of II ($C_{10}H_{10}O_7$).

I. $C_{10}H_{10}O_5$. The metabolic product $C_{10}H_{10}O_5$ resembles orsellinic acid (3:5-dihydroxy-2-carboxytoluene) in many respects, *e.g.* in its ferric chloride reaction, its dibasic nature and its ability to give coloured solutions with excess of alkali. The above constitutional formula shows that $C_{10}H_{10}O_5$ is very closely related to orsellinic acid, being in fact its *C*-acetyl derivative. The fact that $C_{10}H_{10}O_5$ readily loses water on heating or by the action of hot mineral acids to yield the non-ketonic $C_{10}H_8O_4$—a feeble (monobasic) acid not very readily hydrolysed back again to $C_{10}H_{10}O_5$ by alkali—also finds a simple explanation. According to Gottlieb [1899], *o*-carboxybenzylmethylketone is readily converted into 3-methyl*iso*coumarin by the action of hot dilute mineral acids, and on this analogy, $C_{10}H_8O_4$ must be 6:8-dihydroxy-3-methyl*iso*coumarin (IV).



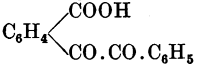
IV. $C_{10}H_8O_4$



V. $C_{12}H_{13}O_3N$

$C_{10}H_8O_4$ should therefore contain two phenolic OH groups like $C_{10}H_{10}O_5$, and there is ample evidence to show that neither of the phenolic OH groups in the latter is concerned in the elimination of H_2O. For example, $C_{10}H_8O_4$ yields a neutral diacetyl derivative which can also be obtained by the acetylation of $C_{10}H_{10}O_5$. $C_{10}H_8O_4$ also yields a neutral non-phenolic dimethyl derivative when methylated by CH_3I and K_2CO_3 in acetone. This derivative can also be obtained by methylation of the methyl ester of the monomethyl ether of $C_{10}H_{10}O_5$ (formed when $C_{10}H_{10}O_5$ is methylated by diazomethane) by the same method. It yields 3:5-dimethoxyphthalic acid on oxidation, and, when hydrolysed by hot alkali, gives a non-phenolic keto-acid $C_{12}H_{14}O_5$ containing two methoxyl groups, evidently 3:5-dimethoxy-2-carboxybenzylmethylketone. The fact that diazomethane methylates only one of the phenolic OH groups of $C_{10}H_{10}O_5$ is doubtless to be explained by the presence of COOH *ortho* to one of the OH groups. In $C_{10}H_8O_4$ this same OH group is still in the *ortho* position to a carbonyl group (hence the feebly acidic properties of $C_{10}H_8O_4$) and it was found also that the methylation of $C_{10}H_8O_4$ even by a tenfold excess of diazomethane yielded a mixture of a feebly acidic monomethyl ether (25 %) together with the dimethyl ether (75 %).

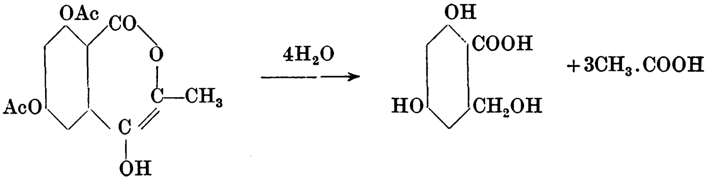
A well known property of *iso*coumarins is that they can be converted into *iso*carbostyril derivatives by heating in a sealed tube with alcoholic ammonia, the —CO—O— being changed into —CO—NH—. The dimethyl ether of $C_{10}H_8O_4$ undergoes a similar transformation, the product $C_{12}H_{13}O_3N$ (V) having the expected properties, being insoluble in alkali and not very readily soluble in dilute HCl.

II. $C_{10}H_{10}O_7$ ($C_{10}H_8O_6, H_2O$). The molecular constitution suggested for $C_{10}H_{10}O_7$ ($C_{10}H_8O_6, H_2O$) presents certain difficulties. Thus $C_{10}H_8O_6$ is represented as an α-diketone and its hydrated form $C_{10}H_{10}O_7$ does in fact condense with *o*-phenylenediamine to give a derivative analysing correctly for the expected quinoxaline, but this crystalline hydrated form, $C_{10}H_{10}O_7$, which was the metabolic product actually isolated, is a colourless substance. The anhydrous substance $C_{10}H_8O_6$, which has not yet been obtained crystalline, has a great affinity for water, being able to abstract it from A.R. chloroform [Clutterbuck, Oxford, Raistrick and Smith, 1932, p. 1454], a fact which suggests that the H_2O is really constitutionally bound to one of the carbonyl groups and that $C_{10}H_{10}O_7$ is in reality only a monoketone (see formula II, second alternative). In support of this view it may be mentioned that $C_{10}H_{10}O_7$ yields only a mono-2:4-dinitrophenylhydrazone (crystallising with $1H_2O$) and even when treated with a large excess of phenylhydrazine yields only the monophenylhydrazone of $C_{10}H_8O_6$, which, however, appears to crystallise from non-hydroxylic solvents without any water of crystallisation. The supposed quinoxaline (p. 643) derived from $C_{10}H_{10}O_7$ is also abnormal, since it crystallises with $1H_2O$ and does not give the expected fluorescence in benzene or alcoholic solution, or any striking coloration with strong sulphuric acid. $C_{10}H_{10}O_7$ (unlike $C_{10}H_{10}O_5$ and $C_{10}H_{10}O_6$) does, however, give a deep yellow solution when dissolved in cold dilute ammonia, so that the ion appears to be coloured although the undissociated acid is colourless. All α-diketones are not coloured substances (*e.g.* *pp'*-diethoxybenzil is colourless, although the compounds $C_6H_5.CO.CO.CH_3$ and  $C_6H_4\begin{smallmatrix}COOH\\CO.CO.C_6H_5\end{smallmatrix}$ (benzil-*o*-carboxylic acid) are definitely yellow), and the true explanation of the colour of most α-diketones is not yet clear [*cf.* Robinson, 1932, p. 31]. $C_{10}H_8O_6$ appears to be another example of an α-diketone with abnormal properties.

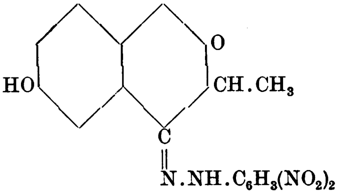
Another fact requiring explanation is the inability, already noted, of $C_{10}H_{10}O_7$ to give a fluorescent solution in cold concentrated sulphuric acid. If the assumption is made that the fluorescence is a property of the *iso*coumarin ring it will be seen that although both $C_{10}H_{10}O_5$ and $C_{10}H_{10}O_6$ are able to lose H_2O to yield *iso*coumarin derivatives, $C_{10}H_8O_6$ cannot yield an *iso*coumarin in this way since in the side-chain —CO.CO.CH_3 there is no hydrogen on the C atom which is directly attached to the benzene ring, and hence $C_{10}H_{10}O_7$ would not be expected to give a fluorescence with concentrated H_2SO_4.

III. $C_{10}H_{10}O_6$. The formula suggested for this acid also presents certain apparent difficulties. Repeated recrystallisation of the pure substance from water undoubtedly lowers the point at which melting begins (see experimental part) and although most specimens of $C_{10}H_{10}O_6$ (as Na salt) reduced copper sulphate in the cold, a specimen of the pure metabolic product isolated *via* the Ca salt and recrystallised from acetone-chloroform (not from water) had no effect on copper sulphate in the cold. Further, although $C_{10}H_{10}O_7$ was obtained by the oxidation of a sample of pure $C_{10}H_{10}O_6$ by excess of copper sulphate the yield was very small and most of the $C_{10}H_{10}O_6$ was recovered unchanged. A possible explanation is offered by the fact that there are 5 possible side-chains (see p. 636) of which only —CHOH.CO.CH_3, —CO.CHOH.CH_3 and —C(OH)=C(OH)CH_3 would be likely to reduce cupric salts in the cold, and that $C_{10}H_{10}O_6$ after crystallisation from water is a mixture of tautomerides. This hypothesis may also explain the optical inactivity of the samples of $C_{10}H_{10}O_6$ isolated, since one possible side-chain, *viz.* —C(OH)=C(OH).CH_3, has no asymmetric carbon atom, and this may be the form in which the substance is actually produced by the mould. It may be recalled that the acetylmethylcarbinol isolated from certain bacterial fermentations is also practically optically inactive.

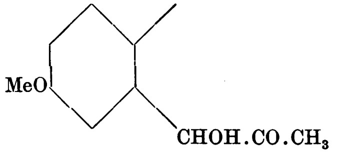
Other properties of $C_{10}H_{10}O_6$ are in good agreement with the suggested structural formula. Acetylation, for instance, yields a neutral acetyl derivative of $C_{10}H_8O_5$ which on hydrolysis yields 3 molecules of acetic acid. It is not quite certain, however, that this derivative is really a triacetyl derivative of $C_{10}H_8O_5$, since the compound obtained when it is hydrolysed by cold alkali appears to have the empirical formula $C_8H_8O_5$. Dieckmann and Meiser [1908] found that *iso*coumarin-4-carboxylic acid was readily split up into formic and homophthalic acids on alkaline hydrolysis so that if the above acetyl derivative is only a diacetyl derivative of $C_{10}H_8O_5$ (the C and H contents of which are practically the same as those of the triacetyl derivative) it might still yield 3 molecules of acetic acid on hydrolysis to give ω-hydroxyorsellinic acid in accordance with the scheme:



Although $C_{10}H_{10}O_6$ (like acetylmethylcarbinol, Clutterbuck, private communication) in aqueous solution does not yield an immediate precipitate with Brady's reagent, the precipitate which slowly forms on long standing analyses correctly for the 2:4-dinitrophenylhydrazone of $C_{10}H_8O_5$ (possibly formula VI). Methylation of $C_{10}H_{10}O_6$ by diazomethane yields a phenolic dimethyl derivative (formula VII).

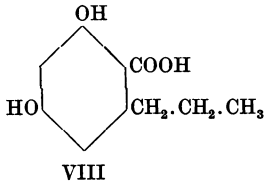


VI



VII

It may be mentioned here that yet another analogy (and the closest so far met with) between mould metabolic products and lichen acids is afforded by comparing the structural unit of the depside divaricatic acid extracted from the lichen *Evernia divaricata* [Hesse, 1911] with the structure proposed for $C_{10}H_{10}O_5$ (I). Divaricatic acid is built up from 2:4-dihydroxy-6-*n*-propylbenzoic acid (VIII) [Hesse, 1911; Mauthner, 1922; 1924; Sonn and Scheffler, 1924; Sonn, 1931; Asahina and Hirakata, 1932] which is theoretically derivable from the mould metabolic product $C_{10}H_{10}O_5$ by the reduction of the $>$CO group in the side-chain of the latter to $>CH_2$.



VIII

Unfortunately, owing to the ease with which water is eliminated from $C_{10}H_{10}O_5$ to yield $C_{10}H_8O_4$, this reduction appears not to be possible *in vitro* by the ordinary Clemmensen method.

EXPERIMENTAL.

Section I.

(*a*) *Separation of $C_{10}H_{10}O_5$ from $C_{10}H_{10}O_7$ and relative yields of these products.* It will be recalled (Part XXIV, p. 1452) that these two metabolic products were precipitated together but that no indication was given in that memoir as to their relative yields. Actually the melting-point and behaviour on further heating of the crude fractions containing these substances indicated that, with one exception, *P. stoloniferum* Thom (Ad. 89), $C_{10}H_{10}O_7$ must be the chief constituent. This was confirmed by large-scale fractional crystallisation from water of some 200 g. of mixed $C_{10}H_{10}O_5 + C_{10}H_{10}O_7$ fractions obtained from all the species worked with, with the exception of *P. stoloniferum*, Ad. 89. The yield of $C_{10}H_{10}O_5$ was only 6 g. (3 %), the rest being $C_{10}H_{10}O_7$ together with a considerable amount of gummy material and a very small amount of $C_{10}H_{10}O_6$. In the case of *P. stoloniferum*, Ad. 89, a specimen of the crude fraction had by no means entirely melted at 135° and effervescence was observed at 140–150°; the melt also resolidified after standing for a few days and then had M.P. 120–165°; hence it was concluded that the crude fraction contained much more $C_{10}H_{10}O_5$ than usual. Fractional crystallisation from water, however, revealed that the fraction contained relatively much $C_{10}H_{10}O_6$ and that this was responsible for the anomalous behaviour of the crude fraction on heating. Reference to Table VI in Part XXIV (p. 1451) will indicate that *P. stoloniferum* Ad. 89, gives a larger yield of crude $C_{10}H_{10}O_6$ fractions than any other species. The chief phenolic metabolic products of most species in the *P. brevi-compactum* series are therefore usually mycophenolic acid (if formed at all) and $C_{10}H_{10}O_7$. The yield of $C_{10}H_{10}O_6$ is rather smaller and the yields of $C_{10}H_{10}O_5$ and $C_8H_6O_6$ are very much smaller than that of $C_{10}H_{10}O_7$. With *P. stoloniferum*, Ad. 89, $C_{10}H_{10}O_6$ appears to be the chief metabolic product.

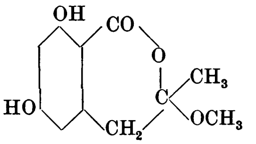
The fractional crystallisation of the mixed $C_{10}H_{10}O_5 + C_{10}H_{10}O_7$ fractions was effected by dissolving in 3 parts of hot water and allowing to stand for several days after seeding with pure $C_{10}H_{10}O_7$. The filtrate from the first crop was allowed to evaporate to about half its volume at room temperature in a vacuum desiccator over calcium chloride. In this way crop 2 and successive crops from successive filtrates were obtained. The first five crops were chiefly $C_{10}H_{10}O_7$, but the last few crops, after seeding with $C_{10}H_{10}O_5$, deposited relatively much $C_{10}H_{10}O_5$. Further purification was effected by dissolving the crude $C_{10}H_{10}O_5$ in a little ethyl acetate and adding boiling 80–100° light petroleum until a marked turbidity was observed and a precipitate began to form. The hot liquid was then filtered through several layers of fluted filter-paper and much boiling 80–100° light petroleum added to the hot filtrate with stirring until precipitation was complete. The almost pure $C_{10}H_{10}O_5$ was filtered off while still hot and a final purification effected by crystallisation from 2 parts of water. The crude $C_{10}H_{10}O_7$ was purified by repeated crystallisation from water (3 parts), the loss being considerable. All the aqueous mother-liquors from the above crystallisations were combined, extracted with ether and the recovered material fractionally crystallised by the same method. This process was repeated as long as the supply of material lasted. No better method of separation was found.

(*b*) *Substance $C_{11}H_{12}O_5$ (probably a methyl derivative of $C_{10}H_{10}O_5$) isolated during the working up of the metabolic products from* P. brevi-compactum *Dierckx* (P. 75). In Part XXIV (p. 1451) it is mentioned that in the working up of fraction VI the use of alcohol must be avoided since it seemed to lead to partial esterification. In the large scale experiments acetone was employed to dissolve the crude fraction, and from the acetone solution $C_{10}H_{10}O_6$ was precipitated by the addition of much boiling chloroform. In some early experiments, methyl alcohol was used instead of acetone, and it was noticed that the final residue contained an abnormally large content of material insoluble in water. Fractional crystallisation of this insoluble residue from chloroform-light petroleum yielded a new substance in fern-like aggregates of colourless needles, M.P. 173–175° without decomposition.

Micro-analysis (Schoeller): C, 58·95; H, 5·36; OCH_3, 13·42 %. $C_{10}H_9O_4(OCH_3)$ requires C, 58·93; H, 5·40; OCH_3, 13·85 %.

0·0801 g. required 3·60 cc. $N/10$ NaOH for neutralisation to phenolphthalein, corresponding to an equivalent of 223. $C_{11}H_{12}O_5$ titrating as a monobasic acid requires an equivalent of 224. The substance is recovered unchanged on acidification of the neutral solution.

The substance is not a true metabolic product, for if acetone is used instead of methyl alcohol, the final residue yields only mycophenolic acid by careful fractional crystallisation from chloroform-light petroleum. $C_{11}H_{12}O_5$ is readily soluble in most organic solvents. It gives an intense but unstable red colour with ferric chloride in alcoholic solution. It is insoluble in water but dissolves immediately in aqueous sodium bicarbonate. It gives no turbidity with Brady's reagent even on standing for an hour, but a copious yellow precipitate forms after standing for a day. The nature of this methyl derivative of $C_{10}H_{10}O_5$ is not quite clear. It appears not to be a ketone, hence is probably not the methyl ester of $C_{10}H_{10}O_5$, but may be a pseudo-ester of the form



[*cf.* Auwers and Heinze, 1919].

(*c*) *Conversion of* $C_{10}H_{10}O_5$ *into* $C_{10}H_8O_4$, *and stability of* $C_{10}H_8O_4$ *towards alkali.* The dehydration of $C_{10}H_{10}O_5$ to give $C_{10}H_8O_4$ is effected:

(i) By heating at 150–170° and crystallisation of the product from water (Part XXIV, p. 1454).

(ii) A more convenient method is to use acetic anhydride as the dehydrating agent and to hydrolyse the resulting diacetyl derivative of $C_{10}H_8O_4$ by cold dilute alkali (see p. 644).

(iii) $C_{10}H_{10}O_5$ is dissolved in water and concentrated H_2SO_4 poured slowly in to make a well-defined bottom layer. A felt of needle-shaped crystals of $C_{10}H_8O_4$ soon forms above the surface of the sulphuric acid.

$C_{10}H_8O_4$ is not readily hydrolysed by $N/40$ NaOH at 70–80°; 0·1146 g. was dissolved in 10 cc. aqueous alcohol and neutralised to phenolphthalein by addition of 5·1 cc. $N/10$ NaOH. (Theoretical for $C_{10}H_8O_4$ titrating as a monobasic acid, 5·95 cc.) A further 5·5 cc. of $N/10$ alkali was added and the solution heated at 70–80° for 40 minutes, when only a further 1·7 cc. was found to have been used up. $C_{10}H_8O_4$ was recovered unchanged by acidifying the solution.

(*d*) *Nature of the compound* $C_{10}H_{10}O_6$ *after recrystallisation from water.* In Part XXIV, p. 1453, it was recorded that this compound melted at 193–203° with softening, darkening and vigorous gas evolution at the latter temperature. Actually the temperature at which softening begins and also the decomposition point seem to vary from one sample to another, *e.g.* a specimen prepared by the catalytic reduction of $C_{10}H_{10}O_7$ (see later) and crystallised once from water did not soften till 202° (decomp. at 206°) while a specimen of the mould metabolic product after five recrystallisations from water began to soften at 178° (decomp. at 200°). Actually this latter material was quite pure and when again recrystallised yielded a crop of crystals, entirely homogeneous when examined under the microscope and melting as above (178–200°). The mother-liquor from this last crystallisation was also evaporated to dryness at room temperature *in vacuo* over H_2SO_4 and yielded material behaving exactly in the same way on heating. In every case the melt reset on cooling and remelted at 190–200° without effervescence. It may be that the rate of heating and other physical factors affect the temperature at which softening begins, but a possible explanation is that the material, after many recrystallisations from water, is a final equilibrium mixture (see introduction, p. 638).

Section II. Presence of keto-groups. Description of tests and derivatives.

(*a*) *Test for the* —$CH_2.CO.CH_2$— *grouping in* $C_{10}H_{10}O_5$, $C_{10}H_{10}O_6$ *and* $C_{10}H_{10}O_7$. The test for the —$CH_2.CO.CH_2$— grouping as described by Täufel and Thaler [1932] consists in the addition of strong sulphuric acid (2 cc.) to a solution of the ketone (a few mg.) in water (4 cc.) which has been shaken with pure salicylaldehyde to form an emulsion. In spite of purification through the bisulphite compound and fractional distillation, the specimen of salicylaldehyde worked with still gave a distinct red coloration in a blank experiment under these conditions, but the following modification of the test was found to give a perfectly satisfactory result with acetone, and to give an almost negative blank. The ketone (a few mg.) is dissolved in cold 45 % (by weight) H_2SO_4 (5 cc.), purified salicylaldehyde (0·5 cc.) is added and the test-tube placed in warm water and shaken vigorously at half minute intervals, the bath being gradually taken up to 100°. In a very few minutes the upper aldehyde layer develops a deep red, and the lower aqueous layer a yellow colour, while in a blank experiment, even after several hours' heating, the aldehyde layer was only faintly pink, and the aqueous layer was colourless. The results obtained with the three metabolic products were as follows: $C_{10}H_{10}O_5$, strongly positive; $C_{10}H_{10}O_6$, faintly positive, *i.e.* rather pale brownish-red, slowly developing; $C_{10}H_{10}O_7$, negative. $C_{10}H_{10}O_5$ therefore very probably contains the —$CH_2.CO.CH_2$— grouping; $C_{10}H_{10}O_7$ does not contain this grouping, while the doubtful positive result given by $C_{10}H_{10}O_6$ may perhaps be explained by the presence of the grouping —$CHOH.CO.CH_3$.

(*b*) *Mono-2:4-dinitrophenylhydrazone of* $C_{10}H_{10}O_5$. This derivative, which is precipitated immediately, was prepared by adding an excess of Brady's reagent (a solution of 2:4-dinitrophenylhydrazine in 2*N* HCl) to a cold saturated aqueous solution of $C_{10}H_{10}O_5$. The yield was, however, much smaller than with $C_{10}H_{10}O_7$ (*ca.* 20 %) and the compound appears to be appreciably soluble in cold water. It forms orange needles from ethyl acetate-light petroleum, much redder in tint than the corresponding derivative of $C_{10}H_{10}O_7$, and melts at 224–226° with blackening and moderately vigorous effervescence. Micro-analysis (Schoeller). Found: loss at 100° *in vacuo*, nil. C, 49·55, 49·44; H, 3·67, 3·74; N, 13·91, 13·96 %. $C_{16}H_{14}O_8N_4$ requires C, 49·21; H, 3·61; N, 14·36 %.

(*c*) *Behaviour of* $C_{10}H_{10}O_6$ *towards 2:4-dinitrophenylhydrazine; formation of 2:4-dinitrophenylhydrazone of* $C_{10}H_8O_5$ (formula VI). A cold saturated aqueous solution of $C_{10}H_{10}O_6$ gave no immediate precipitate when added to excess of Brady's reagent, but turbidity began to appear after about 30 minutes, and a voluminous yellow precipitate had formed after 3 days. This was collected, dried and crystallised from ethyl acetate-light petroleum; it formed balls of needles, darkening above 200° and melting at 232° with vigorous effervescence and blackening. Found (on air-dried material): C, 49·80, 49·63; H, 3·15, 3·32; N, 13·66 %; loss at 100° *in vacuo*, nil. $C_{16}H_{12}O_8N_4$ (dinitrophenylhydrazone of $C_{10}H_8O_5$) requires C, 49·47; H, 3·12; N, 14·4 %.

Evidently $C_{10}H_{10}O_6$ does not itself form a dinitrophenylhydrazone, but is slowly dehydrated by cold 2*N* HCl to yield a ketone $C_{10}H_8O_5$. This dehydration proceeds rapidly on heating. Thus, if a mixture of an aqueous solution of $C_{10}H_{10}O_6$ and Brady's reagent is heated to boiling and then rapidly cooled, a copious precipitate of the above dinitrophenylhydrazone immediately separates.

(*d*) *Mono-2:4-dinitrophenylhydrazone of* $C_{10}H_8O_6$. To a solution of $C_{10}H_{10}O_7$ (0·23 g.) in water (50 cc.) were added 150 cc. of Brady's reagent. A copious, canary-yellow precipitate formed immediately and was later collected (yield 0·39 g. nearly theoretical), and crystallised from ethyl acetate-benzene or ethyl acetate-light petroleum in yellow rhombs or lemon-yellow leaflets, which darken above 200° and melt sharply at 216° with blackening and very vigorous effervescence. Micro-analysis (Schoeller) on air-dried material: Found, loss at 50° *in vacuo*, 8·0, 8·0 %; $C_{16}H_{12}O_9N_4, 2H_2O$ requires for the loss of $2H_2O$, 8·2 %. On anhydrous material; Found, C, 47·29, 47·40; H, 2·98, 3·24; N, 13·18 %. $C_{16}H_{12}O_9N_4$ (dinitrophenylhydrazone of $C_{10}H_8O_6$) requires C, 47·52; H, 3·00; N, 13·87 %. This derivative is sparingly soluble in benzene and chloroform, readily soluble in hot ethyl acetate.

(*e*) *Monophenylhydrazone of* $C_{10}H_8O_6$. (i) *From* $C_{10}H_{10}O_7$. A solution of phenylhydrazine hydrochloride (2·0 g., 3 mols.) and hydrated sodium acetate (6·0 g.) in water (20 cc.) was filtered, and to the filtrate was added a solution of pure $C_{10}H_{10}O_7$ (1·3 g., 1 mol.) in water (10 cc.). The mixture was heated on the steam-bath for an hour, when a dark-coloured oil was slowly deposited, which later partially solidified on cooling and was filtered off. The filtrate when acidified with dilute HCl yielded a copious, orange, crystalline precipitate, which was collected, dried and crystallised from ethyl acetate-light petroleum in bunches of yellow needles (0·5 g.); M.P. 214° decomp. after darkening from 200°. Micro-analysis (Schoeller): loss at 100° *in vacuo*, 1·3, 1·6 %; found on material dried at 100° *in vacuo*: C, 61·20; H, 4·64; N, 8·53 %. $C_{16}H_{14}O_5N_2$ requires, C, 61·13; H, 4·49; N, 8·91 %.

The dark-coloured oil precipitated during the course of the reaction solidified on drying *in vacuo* and was then triturated with dilute HCl. The orange-brown powder so obtained (0·67 g.) appeared to consist essentially of the above compound. Although $C_{10}H_{10}O_7$ ($C_{10}H_8O_6, H_2O$) is the hydrated form of a diketone, it appears to be impossible to convert it into an osazone.

(ii) *From* $C_{10}H_{10}O_6$. The conditions were exactly as above, $C_{10}H_{10}O_6$ (1·2 g.) being used instead of $C_{10}H_{10}O_7$. The course of the reaction was also very similar but the dark-coloured oil deposited was later found to consist chiefly of tarry material. The filtrate on acidification yielded a yellow precipitate, which was crystallised from ethyl acetate-light petroleum (0·53 g.); M.P. 213° decomp., alone or mixed with the phenylhydrazone obtained from $C_{10}H_{10}O_7$ (M.P. 214° decomp.). This fact, together with the analytical figures, shows that the two compounds are identical. Found: loss at 100° *in vacuo*, nil; C, 61·10; H, 4·65; N, 8·06 %. $C_{16}H_{14}O_5N_2$ requires C, 61·13; H, 4·49; N, 8·91 %.

(*f*) *The supposed quinoxaline derived from* $C_{10}H_{10}O_7$. A solution of pure $C_{10}H_{10}O_7$ (1 g.) and *o*-phenylenediamine hydrochloride (0·85 g.; slight excess) in alcohol (15 cc.) was boiled under reflux for 30 minutes. The brown liquid was then poured into much 2*N* H_2SO_4 (no precipitate), and dilute caustic potash solution was then cautiously added with stirring until the maximum amount of precipitate appeared to have been formed. The solution was then still strongly acid. The almost colourless amorphous precipitate was collected and dried (1·3 g.) and crystallised from hot water, in which it is very sparingly soluble, in colourless diamond-shaped platelets; M.P. 210° (effervescence and blackening) with marked softening from 200°. Micro-analysis (Schoeller). On air-dried material, C, 60·31, 60·37; H, 4·60, 4·71; N, 8·92, 8·94 %; mol. wt. 270, 267; loss at 100° *in vacuo*, 5·80, 5·84 %. $C_{16}H_{12}O_4N_2$, H_2O requires C, 61·13; H, 4·49; N, 8·92 %; mol. wt., 314; H_2O, 5·73 %. Found on material dried at 100° *in vacuo*: C, 64·05, 64·18; H, 4·20, 4·24; N, 9·06 %. $C_{16}H_{12}O_4N_2$ requires C, 64·84; H, 4·09; N, 9·46 %.

This derivative is very readily soluble in cold ethyl and methyl alcohol, moderately soluble in hot ethyl acetate and almost insoluble in benzene and chloroform. It is readily soluble in sodium bicarbonate solution and gives a pale yellow colour with caustic soda, but is not readily soluble in dilute sulphuric acid. With concentrated sulphuric acid it gives a warm brown coloration, changing to pale yellow on dilution with water. It gives no precipitate with Brady's reagent, and no fluorescence in alcoholic or benzene solution.

Section III. Acetyl derivatives.

(*a*) *Diacetyl derivative of $C_{10}H_8O_4$.* (i) *From $C_{10}H_{10}O_5$.* A solution of $C_{10}H_{10}O_5$ (0·5 g.) and acetic anhydride (5 cc.) in pyridine (6 cc.) was kept at 36° for 6 days, and was then poured into water (250 cc.). The almost colourless precipitate was filtered off (0·5 g.) and crystallised from light petroleum (80–100°) in which it is moderately readily soluble on heating and much more readily soluble in this and other solvents than the triacetyl derivative of $C_{10}H_8O_5$ (*v. infra*). It formed clusters of colourless needles, M.P. 123–124° without decomposition; no coloration was given with ferric chloride in either aqueous or alcoholic solution. Micro-analysis (Schoeller): C, 61·14, 61·05; H, 4·59, 4·38 %. $C_{14}H_{12}O_6$ requires C, 60·85; H, 4·38 %.

This derivative is slowly but completely soluble in cold caustic soda solution, and acidification of the alkaline solution then gives $C_{10}H_8O_4$ in quantitative yield. This property was made use of to prove that the compound contains two acetyl groups. 0·1087 g. was allowed to stand in contact with 28·95 cc. *N*/10 NaOH with frequent shaking for 3 hours when the whole had completely dissolved. 16·68 cc. *N*/10 H_2SO_4 were then required for neutralisation, hence 12·27 cc. *N*/10 NaOH had been neutralised. The end-point to phenolphthalein also corresponded with the commencement of the precipitation of $C_{10}H_8O_4$. If the hydrolysis yields two molecules of acetic acid and one molecule of the monobasic acid $C_{10}H_8O_4$, 11·82 cc. of *N*/10 NaOH should have been neutralised, while if the hydrolysis yields only one molecule of acetic acid, only 9·29 cc. *N*/10 NaOH would be required. By acidifying the titrated solution and filtering off the precipitate, pure $C_{10}H_8O_4$ (0·065 g.; M.P. 247–249°) was recovered (theoretical, 0·076 g.). The derivative therefore is undoubtedly a diacetyl derivative.

(ii) *From $C_{10}H_8O_4$.* $C_{10}H_8O_4$ (0·1 g.) was acetylated by the above method. The product after one recrystallisation from light petroleum (60–80°) had M.P. 119–121°, and M.P. 119–124° when mixed with the diacetyl derivative, M.P. 123–124° prepared from $C_{10}H_{10}O_5$.

(*b*) *Acetylation of $C_{10}H_{10}O_6$; formation of a supposed triacetyl derivative of $C_{10}H_8O_5$.* To a solution of pure $C_{10}H_{10}O_6$ (1·0 g.) in pyridine (12·5 cc.) acetic anhydride (10 cc.) was slowly added with shaking and cooling. The flask was corked and kept at 37° for 6 days. The deep brownish-red liquid was then poured into water (250 cc.) and the dark-coloured solid precipitate collected and dried (0·97 g., M.P. 138–154°). After crystallisation from a little benzene, followed by two recrystallisations from light petroleum (80–100°) it formed long, pale yellow, lustrous needles, M.P. 172–174° without decomposition.

Found (micro-analysis by Schoeller): C, 57·67, 57·61; H, 4·28, 4·24; CO.CH$_3$ (by hydrolysis with H_2SO_4 and distillation of the acetic acid), 38·1 %; CO.CH$_3$ (by hydrolysis with alcoholic H_2SO_4 and distillation of the ethyl acetate later hydrolysed by standard alcoholic NaOH (A. G. Perkin's method)), 38·4 %. Mol. wt. in camphor, 312, 307, 313; $C_{10}H_5O_2$ (O.CO.CH$_3$)$_3$ (triacetyl derivative of $C_{10}H_8O_5$) requires C, 57·48; H, 4·22; COCH$_3$, 38·62 %. Mol. wt., 334. The substance gives no coloration with $FeCl_3$ and is neutral but is very slowly soluble in cold $N/10$ NaOH. The product so obtained (pale yellow, feathery needles from chloroform-light petroleum) has M.P. 171–176° (but depressed by admixture with the above triacetyl derivative) and gives a purplish-red coloration with ferric chloride. Micro-analysis (Schoeller): C, 51·10; H, 4·55 %. $C_8H_8O_5$ requires C, 52·15; H, 4·38 %. It is possible that this substance is ω-hydroxyorsellinic acid (see introduction, p. 639).

The triacetyl derivative of $C_{10}H_8O_5$ is readily soluble in chloroform and most other organic solvents, but is only moderately soluble in cold benzene and sparingly soluble in hot light petroleum.

Section IV. Methyl derivatives.

(*a*) *Methylation of $C_{10}H_{10}O_5$ by diazomethane; formation of the monomethyl ether of the methyl ester of $C_{10}H_{10}O_5$.* Pure $C_{10}H_{10}O_5$ (0·8790 g.) was treated with a large excess of ethereal diazomethane in two lots, each prepared from 5 cc. of nitrosomethylurethane. Next day, the ether was evaporated leaving an almost colourless, crystalline product (1·02 g.) which gave an intense purple coloration with ferric chloride in alcoholic solution. It was crystallised from light petroleum (60–80°) in which it is moderately soluble even in the cold, and formed feathery groups of colourless needles, M.P. 88–91°. There was no apparent effervescence due to loss of methyl alcohol on slowly raising the temperature to 220°.

Micro-analysis (Schoeller): C, 60·56, 60·60; H, 5·96, 6·02; OCH$_3$, 26·22, 26·31 %; $C_{10}H_8O_3(OCH_3)_2$ requires C, 60·48; H, 5·88; OCH$_3$, 26·05 %.

This dimethyl derivative is insoluble in water and aqueous sodium bicarbonate, but is immediately soluble in dilute caustic potash solution. In aqueous alcoholic solution it gives an immediate copious precipitate with Brady's 2:4-dinitrophenylhydrazine reagent. In alcoholic solution it gives an intense purple coloration with ferric chloride, fading to pale brown in half an hour.

(*b*) *Dimethyl ether of $C_{10}H_8O_4$.* (i) *From the monomethyl ether of the methyl ester of $C_{10}H_{10}O_5$.* A solution of the above dimethyl derivative of $C_{10}H_{10}O_5$ (0·5 g.) and methyl iodide (3 cc.) in acetone (10 cc.) was boiled with finely powdered anhydrous potassium carbonate (3 g.) for 3½ hours. The mixture was then filtered and the filtrate evaporated to dryness *in vacuo*, to yield a partially crystalline brown residue. The crystalline substance was obtained pure by extraction with boiling light petroleum (80–100°) and recrystallisation from the same solvent. It formed clusters of short needles (0·2 g.), M.P. 149–151°.

Micro-analysis (Schoeller): C, 65·24, 65·35; H, 5·41, 5·48; OCH$_3$, 27·74, 27·55 %; $C_{10}H_6O_2(OCH_3)_2$ requires C, 65·42; H, 5·50; OCH$_3$, 28·17 %.

This derivative is much more sparingly soluble in light petroleum than the dimethyl derivative of $C_{10}H_{10}O_5$ from which it was prepared. It gives no coloration with ferric chloride and no precipitate with Brady's reagent in alcoholic solution. It is insoluble in cold and slowly soluble in boiling caustic potash, and decolorises a solution of bromine in chloroform instantly.

(ii) *From* $C_{10}H_8O_4$. A solution of $C_{10}H_8O_4$ (1 g.) and methyl iodide (6 cc.) in acetone (30 cc.) was boiled with anhydrous potassium carbonate (4 g.) as above. The product, isolated in exactly the same way, had M.P. 148–150° alone or mixed with the substance obtained from $C_{10}H_{10}O_5$ by the same method, after one recrystallisation from light petroleum. The yield is much superior (0·7 g.) and if a quantity of the substance is required the best method of preparation is to convert $C_{10}H_{10}O_5$ into $C_{10}H_8O_4$ by acetylation and hydrolysis of the diacetyl derivative by cold alkali (p. 644) and to methylate the crude $C_{10}H_8O_4$ directly as above.

(*c*) *Hydrolysis of the dimethyl ether of* $C_{10}H_8O_4$ *to yield the dimethyl ether of* $C_{10}H_{10}O_5$. 0·0207 g. was boiled under reflux for an hour with 10·00 cc. *N*/10 NaOH. The solution then required 17·9 cc. *N*/20 HCl for neutralisation to phenolphthalein, 2·1 cc. *N*/20 NaOH having been used up (theoretical for the opening of a lactone ring to yield a monobasic acid, 1·9 cc.). The titrated solution was acidified and extracted with ether. Evaporation of the dried ether extract yielded a colourless, crystalline residue, which was recrystallised from light petroleum (80–100°). It formed colourless needles, M.P. 139–141° with slow effervescence at 180°, continuing as the temperature was slowly raised to 220°. Micro-analysis (Schoeller): C, 60·39, 60·52; H, 5·81, 6·06; OCH_3, 26·01, 25·94 %; $C_{10}H_8O_3(OCH_3)_2$ requires C, 60·48; H, 5·88; OCH_3, 26·05 %. 0·1477 g. required 6·22 cc. *N*/10 NaOH for neutralisation to phenolphthalein (sharp end-point) corresponding to an equivalent of 237, the theoretical value for $C_{12}H_{14}O_5$ titrating as a monobasic acid being 238.

This dimethyl ether is but sparingly soluble in high-boiling light petroleum and is partially decomposed by this solvent, the best medium for recrystallisation being chloroform-light petroleum (50–60°). It is sparingly soluble in cold, readily in hot alcohol and in aqueous or alcoholic solution gives no coloration with ferric chloride. It is only moderately soluble in cold ether but readily soluble in cold chloroform. In alcoholic solution it gives an immediate yellow precipitate with Brady's 2:4-dinitrophenylhydrazine reagent. It is immediately soluble in sodium bicarbonate solution.

An attempt to obtain a crystalline oxime failed. The keto-acid (0·0254 g., 1 equiv.) was dissolved in a few cc. of alcohol and hydroxylamine hydrochloride (0·0077 g., 1 equiv.) added together with sufficient *N*/10 NaOH (1·06 cc.) to neutralise all the HCl in the hydroxylamine hydrochloride. The mixture was heated on the water-bath for 30 minutes, allowed to stand for 2 days, then acidified and extracted with ether. Evaporation of the dried ether extract yielded a gum from which nothing crystalline could be obtained.

(*d*) *Methylation of* $C_{10}H_8O_4$ *by diazomethane; formation of a monomethyl ether* $C_{11}H_{10}O_4$. $C_{10}H_8O_4$ (0·85 g.) was treated with a great excess of ethereal diazomethane (3·6 g.; 20 mols.) in two successive portions, each portion being prepared from 10 cc. nitrosomethylurethane. The reaction was very slow, the evolution of nitrogen being scarcely perceptible, and the material dissolved slowly and with difficulty. The reaction mixture was allowed to stand overnight. Next morning, a test portion still gave a positive ferric chloride reaction. The ethereal solution was shaken with dilute sodium carbonate solution until a test portion no longer gave a coloration with ferric chloride. The alkaline extract was acidified with dilute sulphuric acid and the colourless precipitate collected and dried (0·2 g.) and crystallised from light petroleum B.P. 60–80°) in which it is moderately soluble even in the cold. It formed a coherent felt of colourless feathery needles, M.P. 124–125°.

Micro-analysis (Schoeller): C, 64·12, 64·20; H, 4·92, 4·92; OCH_3, 14·38, 14·93 %. $C_{10}H_7O_3(OCH_3)$ requires C, 64·05; H, 4·89; OCH_3 15·04 %.

This derivative is readily soluble in most organic solvents and is sparingly soluble in boiling water. It is insoluble in sodium bicarbonate solution and is only slowly soluble in sodium carbonate solution. It gives an intense and stable purple coloration with ferric chloride in alcoholic solution.

The ethereal solution after shaking out with sodium carbonate was shaken once with water and dried over anhydrous sodium sulphate. Evaporation of the ether yielded a colourless residue, which was crystallised from light petroleum to yield the pure dimethyl ether of $C_{10}H_8O_4$ (0·6 g.), M.P. 150–152° (p. 645).

(*e*) *Methylation of $C_{10}H_{10}O_6$ by diazomethane; formation of a dimethyl derivative, probably the monomethyl ether of the methyl ester.* $C_{10}H_{10}O_6$ (0·874 g.) was treated with a large excess of diazomethane and the reaction mixture allowed to stand overnight. The ether was then removed and the product dried to constant weight in a vacuum desiccator. The yield of crude oily product was 1·0235 g., corresponding to the introduction of 2·7 methyl groups. Partial crystallisation occurred after long standing, and the crystalline product was obtained pure by extraction with light petroleum (60–80°) and repeated recrystallisation from low-boiling light petroleum (50–60°). It formed rosettes of colourless, light, feathery needles (19 mg.), M.P. 82°. With alcoholic ferric chloride it gave a pale purplish-brown coloration, fading in a few minutes to brown, finally to orange-brown.

Micro-analysis (Schoeller): C, 56·90; H, 5·49 %; OCH_3, 24·17, 24·14 %. $C_{10}H_8O_4(OCH_3)_2$ requires C, 56·70; H, 5·55; OCH_3, 24·41 %.

This derivative is insoluble in cold water and cold $NaHCO_3$ solution, but is readily soluble in cold caustic soda.

By the working up of mother-liquors, various syrupy, uncrystallisable residues were obtained as by-products during the isolation of the above dimethyl derivative. These all contained about 22–24 % OCH_3, and were probably mixtures of mono-, di- and trimethyl derivatives of $C_{10}H_{10}O_6$.

Methylation of $C_{10}H_{10}O_7$ by the above method yielded a yellow syrup from which nothing crystalline could be isolated, nor could any crystalline derivative be obtained by further methylation of this syrup by the CH_3I-K_2CO_3-acetone method (see p. 645).

Section V. Interconversion of the three acids $C_{10}H_{10}O_5$, $C_{10}H_{10}O_6$, $C_{10}H_{10}O_7$.

(*a*) *Conversion of $C_{10}H_{10}O_7$ into $C_{10}H_{10}O_6$ by catalytic reduction.* Pure $C_{10}H_{10}O_7$ (0·66 g.; entirely freed from $C_{10}H_{10}O_6$ by six recrystallisations from water) was dissolved in a few cc. of water and added to a solution of palladous chloride (0·2 g.) in dilute acetic acid (50 cc.). Norite (1 g., previously strongly heated) was added and a rapid stream of hydrogen passed in during 2 hours, the flask being vigorously shaken by hand from time to time. The mixture was then filtered and the filtrate made strongly acid with dilute sulphuric acid and extracted with ether. The norite residue was also extracted with ether and the combined ether extracts were dried over anhydrous Na_2SO_4. Evaporation of the ether yielded $C_{10}H_{10}O_6$ in almost quantitative yield, M.P. (after crystallisation from water) 202–206° efferv., and when mixed with authentic $C_{10}H_{10}O_6$ (M.P. 202–204° efferv.) the melting-point was 202–204° (efferv.). Found: C, 53·29, 53·09; H, 4·52, 4·51 %. $C_{10}H_{10}O_6$ requires C, 53·07; H, 4·46 %.

The above figures together with the properties of the compound prove beyond all doubt that it is identical with the metabolic product $C_{10}H_{10}O_6$. Thus it gives a transient red colour with ferric chloride in aqueous solution, but a stable reddish-purple colour in alcoholic solution. Its sodium salt reduces copper sulphate quickly in the cold. In dilute solution it gives no turbidity with Brady's reagent except after standing for more than an hour. Its pale green solution in cold concentrated sulphuric acid has a blue fluorescence in ultra-violet light and becomes deep purplish-brown on heating.

(*b*) *Conversion of $C_{10}H_{10}O_6$ into $C_{10}H_{10}O_7$ by oxidation of the Na salt by copper sulphate in the cold.* $C_{10}H_{10}O_6$ (0·43 g.; many times recrystallised from H_2O) was neutralised by the addition of 30 cc. $N/10$ NaOH. 7 % aqueous copper sulphate solution (24 cc.) was added and the mixture allowed to stand for an hour. Copious precipitation of Cu_2O took place almost immediately, but at the end of an hour, the supernatant liquid was still green in colour and the reaction appeared to have gone to completion. The reaction mixture was filtered, the filtrate acidified with dilute sulphuric acid and extracted with ether. Evaporation of the dried ether extract yielded a pale yellow crystalline solid, most of which was insoluble in boiling chloroform and indeed proved to be unchanged $C_{10}H_{10}O_6$. The hot chloroform extract was diluted with boiling petroleum (60–80°) until a pronounced turbidity was observed and put aside to crystallise, yielding a small amount of pale yellow, flat needles, M.P. 110–132°, with vigorous efferv., raised to 120–133° (efferv.) after one crystallisation from water; yield 0·01 g. Found: C, 48·78; H, 4·39 %. $C_{10}H_{10}O_7$ requires C, 49·57; H, 4·17 %. The substance gave an immediate precipitate with Brady's reagent and was undoubtedly $C_{10}H_{10}O_7$.

Because of the smallness of the yield it was necessary to prove that the original $C_{10}H_{10}O_6$ contained no $C_{10}H_{10}O_7$. 0·5 g. of the same specimen of $C_{10}H_{10}O_6$ was extracted with 200 cc. boiling chloroform, but the minute amount of oily material obtained by evaporation of the chloroform extract gave no immediate precipitate with Brady's reagent. $C_{10}H_{10}O_7$ is therefore a true oxidation product of $C_{10}H_{10}O_6$. The smallness of the yield may be accounted for by the suggestion previously put forward (p. 642) that $C_{10}H_{10}O_6$ after many recrystallisations from water forms a stable equilibrium mixture. If one component only is readily oxidised under the above conditions and the equilibrium is re-established very slowly in the cold, part of the $C_{10}H_{10}O_6$ would be recovered unchanged although the reaction appeared to have proceeded to completion.

(*c*) *Conversion of $C_{10}H_{10}O_7$ and $C_{10}H_{10}O_6$ into $C_{10}H_{10}O_5$ by reduction by zinc dust and dilute sulphuric acid.* Pure $C_{10}H_{10}O_7$ (2·83 g., 6 times recrystallised from H_2O) was dissolved in dilute sulphuric acid (100 cc. of $2N$) by heating, and a few cc. of the solution removed for a control experiment to determine the stability of $C_{10}H_{10}O_7$ towards hot dilute sulphuric acid (actually $C_{10}H_{10}O_7$ was recovered unchanged after boiling the test portion for 3 hours). The bulk of the liquid was maintained at 80–90° while a very large excess of zinc dust was added in small lots with frequent shaking. The whole was then heated on the steam-bath for 2 hours, cooled and filtered. The residual zinc was washed first with dilute sulphuric acid and then several times with water, and the combined filtrates were extracted 5 times with much ether. The dried ethereal extract was evaporated to small bulk and poured into much light petroleum when a pale brown oil separated which later solidified (yield of crude $C_{10}H_{10}O_5$, *ca.* 20 %). After one crystallisation from water (2 parts) it yielded pure $C_{10}H_{10}O_5$ in large colourless tablets, M.P. 150–160° (vigorous efferv.) resets and remelts at 210–222° with slight darkening.

The same product was obtained in rather smaller yield when $C_{10}H_{10}O_6$ was reduced under the same conditions. In this instance, several crystallisations from ethyl acetate-light petroleum followed by one crystallisation from water were needed to effect complete purification (the chief reduction product being an uncrystallisable gum). The substance melted at 152–156° (efferv.) alone or mixed with the reduction product from $C_{10}H_{10}O_7$, or with the authentic metabolic product $C_{10}H_{10}O_5$. In every instance the melt reset and remelted at 210–230°. Found: C, 57·20, 57·06; H, 4·86, 4·90 %. $C_{10}H_{10}O_5$ requires C, 57·14;

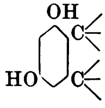
H, 4·76 %. In order to place the matter beyond doubt, the products obtained by heating the two reduction products at 150–170° were each recrystallised from water to give colourless needles, M.P. 242–248°, in each instance, either alone or mixed with an authentic specimen of $C_{10}H_8O_4$, M.P. 244–248°. Micro-analysis (Schoeller) on dehydration product from reduction product of $C_{10}H_{10}O_7$; found: C, 61·89; H, 4·61 %. $C_{10}H_8O_4$ requires C, 62·50; H, 4·20 %.

The zinc dust residues from the reduction of $C_{10}H_{10}O_7$ yielded, by dissolution in dilute sulphuric acid and extraction with ether, a small amount of $C_{10}H_8O_4$.

(*d*) *Reduction of $C_{10}H_{10}O_7$ and $C_{10}H_{10}O_5$ by Clemmensen's method: formation of $C_{10}H_8O_4$.* $C_{10}H_{10}O_7$ (3 g.; freed from $C_{10}H_{10}O_5$ by 5 recrystallisations from water) was dissolved in 350 cc. 12 % hydrochloric acid and added to amalgamated zinc (300 g.). The whole was boiled under reflux for 3½ hours. During the course of the reduction a considerable amount of colourless material sublimed and condensed on the lower surface of the condenser cork. This was scraped off and crystallised from water and proved to be $C_{10}H_8O_4$ (M.P. 230–247° alone or mixed with authentic $C_{10}H_8O_4$). The aqueous reaction liquid was decanted from the zinc and extracted with ether. Evaporation of the ether yielded a brown semi-solid product which was treated with boiling water (500 cc.). The aqueous extract deposited, on cooling, impure $C_{10}H_8O_4$ mixed with tarry material. The sandy residue unextracted by water was crystallised from much water yielding well formed hexagonal prisms, M.P. 234–248°, alone or mixed with authentic $C_{10}H_8O_4$. The total yield of $C_{10}H_8O_4$ was small, but no other crystalline product could be isolated.

The reduction of $C_{10}H_{10}O_5$ (2 g. crystallised from H_2O to remove $C_{10}H_{10}O_7$) was carried out under similar conditions but the method of working up the crude product was somewhat modified. The hot supernatant liquid was decanted and the zinc was washed with a little water, the washings being added to the supernatant liquid. To this was also added the deposit on the cork, and the whole was placed in the ice-chest overnight. The copious, colourless crystalline precipitate was collected (0·18 g.) and crystallised from water; M.P. 240–248° (darkening) alone or mixed with authentic $C_{10}H_8O_4$. When mixed with the product, M.P. 234–248°, obtained by the Clemmensen reduction of $C_{10}H_{10}O_7$, the M.P. was 236–248°. Found: C, 62·33, 62·39; H, 4·35, 4·30 %. $C_{10}H_8O_4$ requires C, 62·50; H, 4·20 %. The aqueous filtrate from the above and also the residual zinc were extracted with ether and yielded 1·18 g. of a partly crystalline paste from which $C_{10}H_8O_4$, but no other crystalline material, was isolated.

Section VI. Proof that the three acids contain the structure



(*a*) *Oxidation of $C_{10}H_{10}O_7$ by H_2O_2 in alkaline solution; formation of 3:5-dihydroxyphthalic acid.* Pure $C_{10}H_{10}O_7$ (0·3 g.) was dissolved in a few cc. of sodium bicarbonate solution and a very large excess of hydrogen peroxide solution added. The deep brown solution was heated on the water-bath for about an hour, acidified and extracted once with ether. Evaporation of the dried ether extract yielded a dark oil, which was dissolved in a little ethyl acetate and much benzene added. After standing for several days, a little brown crystalline material had been deposited, which after crystallisation from ethyl acetate-light petroleum yielded a minute amount of almost colourless crystalline material (plates), M.P. 192° (efferv.), reset and remelted at 200–206°. Mixed with authentic 3:5-dihydroxyphthalic acid the M.P. was 190–192° (efferv.) reset and remelted at

200–208°. The oxidation product, which gave a deep slightly purplish-red coloration with ferric chloride in aqueous solution is therefore undoubtedly 3:5-dihydroxyphthalic acid.

(*b*) *Oxidation of the dimethyl ether of $C_{10}H_8O_4$ by cold alkaline $KMnO_4$; formation of 3:5-dimethoxyphthalic acid.* The pure dimethyl ether of $C_{10}H_8O_4$ (0·05 g.) was suspended in 10 cc. *N* NaOH and 5 % aqueous potassium permanganate solution added, at first drop by drop with shaking (the purple colour being quickly destroyed), later in large excess with occasional shaking. After filtration from MnO_2, decoloration of the filtrate by SO_2, extraction with ether and evaporation of the dried ether extract, a colourless solid product was obtained which was dissolved in a little hot water and the aqueous solution allowed to stand in the ice-chest overnight. Small colourless prisms separated (0·02 g.), which were dried in air, and their behaviour on heating was carefully observed. Two crystals placed in a melting-point tube became opaque between 100 and 130°, but nothing further happened till 180–182° was reached, when effervescence took place. The temperature was taken to 194°, and the tube then removed from the bath when the melt immediately reset and then remelted at 142–148°. The recorded decomposition-point of 3:5-dimethoxyphthalic acid to give the anhydride is 158° [Oxford and Raistrick, 1932], the second melting-point, *i.e.*, of the anhydride, being 143–148°. In order to clear up this discrepancy the behaviour of a single crystal of authentic 3:5-dimethoxyphthalic acid on heating was noted, when it was observed that no effervescence took place till 184° was reached, although when a melting-point was taken in the ordinary way using a column of substance about 2 mm. deep in the melting-point tube, effervescence did occur at 158°. The melting-point of a mixture of the oxidation product with authentic 3:5-dimethoxyphthalic acid, the temperature being slowly raised, was 160–162° (efferv.) the second melting-point being 144–149°. A second mixture had M.P. about 160° (efferv.), the second melting-point being 142–148°. There can thus be no doubt that the oxidation product is in fact 3:5-dimethoxyphthalic acid, a conclusion confirmed by a complete micro-analysis (Schoeller). Found: loss at 100° *in vacuo* 8·2, 8·1 %; $C_8H_4O_4(OCH_3)_2, H_2O$ requires H_2O, 7·4 %. Found (on anhydrous material); C, 53·36, 53·31; H, 4·66, 4·54; OCH_3, 26·77 %. $C_8H_4O_4(OCH_3)_2$ requires C, 53·08; H, 4·45; OCH_3, 27·41 %.

The same substance was obtained in smaller yield when the oxidation by alkaline permanganate was carried out at 100°.

(*c*) *Action of heat on the dimethyl ether of $C_{10}H_{10}O_5$. Formation of a neutral ketone yielding 3:5-dimethoxybenzoic acid on oxidation by alkaline permanganate.* The dimethyl ether of $C_{10}H_{10}O_5$ ($C_{12}H_{14}O_5$) (0·3310 g.) was heated in a slow stream of dry and CO_2-free N_2 at 180–210° until effervescence had ceased. The gaseous reaction products were passed first through a weighed $CaCl_2$ U-tube and then through two bubblers each containing 20 cc. *N*/5 baryta. The water given off weighed 0·0250 g. (0·9 equivalent) and the CO_2 given off was equivalent to 3·3 cc. *N*/10 baryta (*i.e.* 0·0073 g.; 0·1 equivalent). The melt, which was dark in colour and reset on cooling, weighed 0·2977 g. so that the loss in weight was 0·0333 g. while the combined weights of H_2O and CO_2 absorbed totalled 0·0323 g. The melt was essentially the dimethyl ether of $C_{10}H_8O_4$ and on hydrolysis by means of boiling *N*/10 NaOH yielded $C_{12}H_{14}O_5$ identical in all respects with the starting material. It was noticed, however, that a small amount of colourless oil had sublimed and condensed on the cool part of the tube in which the heating had been carried out. The top part of the tube containing this oil was therefore cut off and the oil dissolved in water. To a small portion of the solution was added an excess of 2:4-dinitrophenylhydrazine reagent when an immediate turbidity, later changing to a yellow precipitate was produced. The rest of the solution was made alkaline and several cc. of 5 % $KMnO_4$ solution were added. After standing for several hours, the liquid was decolorised by SO_2 and extracted with ether. Evaporation of the dried ether extract yielded a minute amount of a pale yellow solid which was crystallised from water to yield a few ill-defined crystals, M.P. 174–178°, with partial sublimation in the form of colourless needle-shaped crystals which melted sharply at 178–180°.

The experiment was repeated starting from 0·8 g. of $C_{12}H_{14}O_5$. The sublimate on the cool part of the tube was dissolved in water as before, and the melt was hydrolysed by boiling with excess of $N/10$ NaOH for an hour. The hydrolysis liquid was then made strongly alkaline and extracted twice with ether, the residual aqueous solution being then acidified and extracted with ether for the recovery of $C_{12}H_{14}O_5$. The recovered substance, after crystallisation from chloroform-light petroleum, was heated and the products worked up as before. After 4 such heatings, the weight of recovered $C_{12}H_{14}O_5$ was only 0·13 g. Oxidation of the combined aqueous solutions of the oily sublimate by alkaline permanganate yielded only a trace of material which sublimed at about 200° to yield needles of M.P. 176–178°. The combined ether extracts of the alkaline hydrolysis solutions were evaporated to yield a small amount of a yellow oil, which was dissolved in much water and filtered from a little insoluble material. A portion of this solution when treated with Brady's reagent yielded a 2:4-dinitrophenylhydrazone, M.P. 131–132° after crystallisation from ethyl acetate-light petroleum, which was insoluble in dilute alkali and hence could not have been the 2:4-dinitrophenylhydrazone of the starting material $C_{12}H_{14}O_5$. The remainder of the aqueous solution of the neutral ketone was oxidised by alkaline permanganate in the cold, and yielded about 1 mg. of a substance (after crystallisation from water) which was readily soluble in sodium bicarbonate solution, and which melted with partial sublimation at 174–176°, the sublimate (colourless needles) melting at 178–179°. A mixture of this product with an authentic specimen of 3:5-dimethoxybenzoic acid (M.P. 181–182°; sublimate melting at 181°) melted at 176–182°, the sublimate melting at 180°.

It is thus obvious that in the molecule of $C_{10}H_{10}O_5$, and hence also in $C_{10}H_{10}O_6$ and $C_{10}H_{10}O_7$, the carboxyl group is attached directly to the benzene ring. Also, since the oxidation of the neutral ketone obtained by the decarboxylation of the dimethyl ether of $C_{10}H_{10}O_5$ yields 3:5-dimethoxybenzoic acid the 3-carbon ketonic side-chain must be *meta* to the phenolic OH groups in these acids. In the light of evidence previously produced this side-chain must be in the *ortho* position to the carboxyl group attached directly to the benzene ring.

(*d*) *Behaviour of the dimethyl ether of $C_{10}H_{10}O_5$ towards alkaline iodine* [cf. *Birkinshaw and Raistrick, 1931*]. *Proof of the presence of the side-chain —$CH_2.CO.CH_3$.* The pure ether (11·3 mg.) was dissolved in a few cc. of $N/10$ NaOH, and water (70 cc.) added, followed by $N/10$ iodine (40·0 cc. accurately measured from a pipette) and $N/10$ NaOH (50 cc.). The flask was corked and allowed to stand. A turbidity was noticed within 5 minutes and precipitation of iodoform appeared to be complete within 30 minutes. At the end of 2 hours, $2N$ H_2SO_4 (10 cc.) was added and the unused iodine titrated against $N/10$ thiosulphate when it was found that 4·47 cc. $N/10$ iodine had been used up, corresponding to 9·4 atoms of iodine per molecule of $C_{12}H_{14}O_5$. The yellow crystalline precipitate was filtered off and was undoubtedly iodoform, having M.P. 116–120°.

Similar experiments were carried out with propiophenone (redistilled B.P. 215–216°) and with benzylmethylketone (redistilled B.P. 214–216°). The first named ketone (9·8 mg.) oxidised under conditions exactly similar to those outlined above, used up only 1·48 cc. *N*/10 iodine (*i.e.* 2·0 atoms per molecule of propiophenone) and no trace of iodoform or any other insoluble material was formed. The parallel experiment with benzylmethylketone was carried out under the following conditions. The ketone (8·4 mg.) was dissolved in water (20 cc.) and *N*/10 iodine (60·0 cc.) was added, followed by *N*/10 NaOH (75 cc.). The mixture was allowed to stand for 2½ hours, but separation of iodoform appeared to be complete within the first 30 minutes. 5·49 cc. *N*/10 iodine were used up, corresponding to 8·8 atoms of iodine per molecule of ketone. Since the formation of iodoform and the substituted acetic acid from a substituted acetone requires only 6 atoms of iodine, it is evident that both with $C_{12}H_{14}O_5$ and with benzylmethylketone the oxidation proceeds beyond this stage, probably to the corresponding benzoic acid if a sufficiently large excess of iodine is allowed to act on the ketone for a long enough time. In a control experiment with 3:5-dimethoxyphthalic acid hydrate (14·2 mg.) under the conditions described above for the oxidation of $C_{12}H_{14}O_5$, only 0·08 cc. *N*/10 iodine had been used up in the course of 2 hours, and no iodoform was produced.

It is thus very probable that the 3-carbon side-chain in $C_{10}H_{10}O_5$ is $—CH_2.CO.CH_3$ as in benzylmethylketone and not $—CO.CH_2.CH_3$ as in propiophenone.

(*e*) *Conversion of the dimethyl ether of* $C_{10}H_8O_4$ ($C_{12}H_{12}O_4$) *into* $C_{12}H_{13}O_3N$, *presumably 6:8-dimethoxy-3-methyl*iso*carbostyril* (*Formula V*) [cf. *Bamberger, 1892*]. A mixture of $C_{12}H_{12}O_4$ (0·4 g.) and saturated ethyl alcoholic ammonia (13 cc.) was heated in a sealed tube for 4 hours at 120–160°. The alcohol was then removed by evaporation, finally *in vacuo*, leaving a brown, solid product which was dissolved in a little cold chloroform, and boiling light petroleum (60–80°) was added until a pronounced flocculent precipitate had formed. The hot solution was then filtered and more hot light petroleum added to the filtrate. Fan-shaped clusters of needles soon began to separate, which when collected and dried in air became slightly brown in colour; M.P. 216–218° without decomposition.

Micro-analysis (Schoeller): C, 65·24, 65·46; H, 6·01, 6·02; N, 6·43, 6·51; OCH_3, 27·51 %. $C_{10}H_7ON(OCH_3)_2$ requires C, 65·72; H, 5·98; N, 6·39; OCH_3, 28·30 %.

This substance is readily soluble in cold chloroform, moderately soluble in hot water, only sparingly soluble in cold ethyl alcohol or acetone, and practically insoluble in hot light petroleum and cold water. The aqueous solution gives no coloration with ferric chloride. The substance is a feeble base, dissolving slowly in cold, dilute hydrochloric acid, but is quite insoluble in cold caustic soda solution. It gives no coloration with cold concentrated sulphuric acid, but a deep red colour develops on adding a trace of potassium dichromate.

SUMMARY.

The three acid metabolic products, containing ten carbon atoms, of *P. brevicompactum* Dierckx and related species, *viz.* $C_{10}H_{10}O_5$, $C_{10}H_{10}O_6$, and $C_{10}H_{10}O_7$ have been shown to be 3:5-dihydroxy-2-carboxybenzylmethylketone, 3:5-dihydroxy-2-carboxyphenylacetylcarbinol (or a mixture of its tautomerides) and a hydrated form of 3:5-dihydroxy-2-carboxybenzoylmethylketone respectively. They are therefore closely related constitutionally to each other, to 3:5-dihydroxyphthalic acid and to mycophenolic acid (other metabolic products of the same series of fungi), and to the lichen acid divaricatic acid.

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