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Studies in the Biochemistry of Micro-organisms

100. METABOLITES OF *PENICILLIUM ATROVENETUM* G. SMITH. PART I. ATROVENETIN, A NEW CRYSTALLINE COLOURING MATTER*

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Penicillium atrovenetum G. Smith is a new species which was described recently by Mr George Smith of this Department (1956). Its specific name was given because colonies of the mould on Czapek agar are at first bright bluish green (*venetus*) in colour but rapidly turn greyer and become almost black (*ater*) in age. The reverse of the colonies is a deep yellowish brown. This species is closely related morphologically to *P. herquei* Bainier & Sartory and should therefore be placed in the *P. herquei* series of the Biverticillata-Symmetrica as described by Raper & Thom (1949). Smith (1956) proposes, however, that a better placement of the *P. herquei* series is in the Asymmetrica-Velutina in close association with the *P. citrinum* and *P. brevi-compactum* series.

During the routine chemical examination of a large number of species of moulds it was found here that the mycelium of the type strain and also of two different strains of *P. atrovenetum*, grown on Czapek–Dox glucose–mineral salts solution, produced some interesting reactions. Immersion of portions of the pressed mycelium in ethanol yielded an orange-brown extract which gave an intensely dark-olive brown, almost black ferric reaction. Other portions immersed in cold concentrated sulphuric acid gave an immediate yellow to orange-brown solution with a strong green fluorescence. We have isolated the fungal colouring matter which is responsible for these colour reactions and, since it has not been described previously, we propose for it the name atrovenetin.

For the bulk preparation of atrovenetin *P. atrovenetum* strain S.M. 683 was cultivated on Czapek–Dox glucose solution for 21 days at 24° in the dark. The mould mycelium was then separated, washed with water, dried *in vacuo* and ground to a fine powder. The powder was extracted with light petroleum to remove 'fat' and then with ether. From the ether extracts, initially very dark green in colour, crude atrovenetin separated as a dark powder from which much of the dark material was separated by extraction with acetone. The residual yellow-brown colouring matter was purified by crystallization from dioxan or acetone. The yield of crystalline atrovenetin obtained averaged about 23 g. from 100 flasks containing 35 l. of Czapek–Dox solution and constituted about 43 % of the total ether extract. The combined weights of the light petroleum and ether extracts amounted to 18–20 % of the dry weight of the mould mycelium.

Atrovenetin, $C_{19}H_{18}O_6$, forms yellow-brown plates or prisms, which melt with decomposition at 295° and sublime in high vacuum at about 210°. Solutions of atrovenetin in dioxan are strongly dextrorotatory. The molecule of atrovenetin contains a minimum of four hydroxyl groups, no methoxyl group and at least three or probably four methyl groups attached to carbon. It is not readily soluble in the usual organic solvents. It is not soluble in sodium carbonate or sodium bicarbonate solution but dissolves readily in aqueous sodium hydroxide to an orange solution. Its solution in cold concentrated sulphuric acid is bright yellow in colour with a characteristic intense yellow-green fluorescence. It also gives a characteristic intense red-brown ferric reaction in ethanol solution.

The following functional derivatives of atrovenetin have been prepared: atrovenetin hydrochloride, $C_{19}H_{18}O_6.HCl$, yellow needles, m.p. 285–286° (decomp.); atrovenetin perchlorate,

$$C_{19}H_{18}O_6.HClO_4,2H_2O,$$

* Part 99: Birkinshaw & Hammady (1957).

yellow needles, m.p. 227° (decomp.); triacetylatrovenetin, $C_{19}H_{15}O_3.(O.CO.CH_3)_3$, orange-brown cubes, m.p. 187–188°, dextrorotatory and giving a red-brown ferric reaction.

Several methyl ethers of atrovenetin have been prepared, including a number of isomers which are clearly of importance in considering its molecular constitution. They are:

(i) Atrovenetin monomethyl ether A,

$$C_{19}H_{17}O_5(OCH_3),$$

orange-red plates, m.p. 262–263°, which gives a diacetate, $C_{19}H_{15}O_3(OCH_3).(O.CO.CH_3)_2$, golden needles, m.p. 218–219°, and a diethyl ether, $C_{19}H_{15}O_3(OCH_3).(OC_2H_5)_2$, yellow needles, m.p. 129–130°. All three compounds are dextrorotatory and give intense red-brown ferric reactions, but whereas the monomethyl ether A is soluble in aqueous 2N sodium hydroxide, its diacetate and its diethyl ether do not dissolve in this reagent.

(ii) Atrovenetin monomethyl ether B,

$$C_{19}H_{17}O_5(OCH_3),$$

yellow plates, m.p. 226–227°, soluble in aqueous sodium bicarbonate and giving a green ferric reaction.

(iii) Atrovenetin yellow trimethyl ether, $C_{19}H_{15}O_3(OCH_3)_3$, yellow plates, m.p. 168–169°, which gives a ferrichloride, $C_{22}H_{24}O_6.HFeCl_4$, red-orange rhombs, m.p. 148° (decomp.), and a monoacetate perchlorate,

$$C_{19}H_{14}O_2(OCH_3)_3.(O.CO.CH_3).HClO_4,$$

orange-yellow plates, m.p. 185° (decomp.), which does not give a ferric reaction. The parent yellow trimethyl ether is readily soluble in concentrated hydrochloric acid.

(iv) Atrovenetin orange trimethyl ether, $C_{19}H_{15}O_3(OCH_3)_3$, orange plates, m.p. 178–179°, which gives a ferrichloride, $C_{22}H_{24}O_6.HFeCl_4$, red-orange rhombs, m.p. 151–152°, lowered to 125–130° on admixture with the ferrichloride of atrovenetin yellow trimethyl ether, m.p. 148°. Both the trimethyl ethers are dextrorotatory, insoluble in aqueous sodium hydroxide and give an intense red-brown ferric reaction.

(v) Atrovenetin tetramethyl ether A,

$$C_{19}H_{14}O_2(OCH_3)_4,$$

yellow needles, m.p. 141–143°, dextrotatory. It is readily hydrolysed to atrovenetin orange trimethyl ether by boiling with dilute ethanolic hydrochloric acid.

(vi) Atrovenetin tetramethyl ether B,

$$C_{19}H_{14}O_2(OCH_3)_4,$$

yellow prisms, m.p. 186–187°. Both the tetramethyl ethers are insoluble in aqueous sodium hydroxide, but whereas the tetramethyl ether A gives no ferric reaction, the tetramethyl ether B gives a very pale-brown ferric colour.

A number of degradation experiments have been carried out on atrovenetin and on some of its functional derivatives, the results of some of which indicate that atrovenetin is an unexpectedly stable substance. Thus it was recovered substantially unchanged when it was boiled in an atmosphere of nitrogen for a long time either with aqueous 2N sulphuric acid or with aqueous N potassium hydroxide. It also proved stable towards boiling dilute methanolic sulphuric acid, and its yellow and orange trimethyl ethers similarly resisted the action of boiling 10% methanolic hydrochloric acid or 10% methanolic potassium hydroxide. Even when atrovenetin was fused with potassium hydroxide at 300° for 15 min., 60% of it was recovered unchanged. This behaviour is in marked contrast to that of two other fungal colouring matters, herqueinone and norherqueinone, to which, as will be shown later, atrovenetin is related chemically. Norherqueinone, $C_{19}H_{18}O_7$, and its monomethyl ether herqueinone, $C_{20}H_{20}O_7$, have recently been isolated from strains of *Penicillium herquei* Bainier & Sartory (Stodola, Raper & Fennell, 1951; Harman, Cason, Stodola & Adkins, 1955; Galarraga, Neill & Raistrick, 1955). Both these substances are readily decomposed with boiling aqueous 2N sulphuric acid giving one molecule of methyl *iso*propyl ketone, $C_5H_{10}O$, from each substance, together with one molecule of norxanthoherquein, $C_{14}H_{10}O_7$, from norherqueinone and one molecule of xanthoherquein, $C_{15}H_{12}O_7$, from herqueinone. Alkaline hydrolysis of herqueinone leads to deep-seated decomposition, acetaldehyde being the only recognizable decomposition product.

Attempts to dehydrogenate atrovenetin yellow trimethyl ether either with a palladium–charcoal catalyst in boiling Dowtherm in an atmosphere of carbon dioxide or with chloranil in boiling xylene failed, as also did attempts to reduce the same ether either with sodium in boiling ethanol or catalytically with palladium–charcoal–hydrogen. The ether was recovered substantially unchanged in all cases. Reduction experiments with lithium aluminium hydride in ether proved more successful, the two isomeric trimethyl ethers of atrovenetin, $C_{22}H_{24}O_6$, giving different but isomeric reduction products, $C_{22}H_{24}O_5$, i.e. yellow needles, m.p. 145–146°, from the yellow trimethyl ether and scarlet plates, m.p. 203–204°, from the orange trimethyl ether. Neither of the $LiAlH_4$ reduction products gives a positive ferric reaction in ethanol, in contrast to the intense red-brown ferric reactions of the parent methyl ethers.

Treatment of atrovenetin with alkaline hydrogen peroxide at room temperature gave two colourless oxidation products: *A*, $C_{15}H_{16}O_7$, needles, m.p. 188°,

which titrated as a dibasic acid and gave only a faint-brown ferric reaction; *B*, $C_{12}H_8O_7$, needles, m.p. 151–152°, which titrated as a tribasic acid and gave a red-brown ferric reaction. Neither product gave a precipitate with Brady's reagent. Oxidation of atrovenetin with concentrated nitric acid at 100° gave a product, probably $C_{15}H_{14}O_9N_2 + H_2O$, cream rectangular plates, m.p. 234–235°, which, on treatment with diazomethane, gave a monomethyl derivative, probably $C_{16}H_{16}O_9N_2$, cream plates, m.p. 251°. Both these substances are dextrorotatory and both give a negative ferric reaction in ethanol. Only oxalic acid was isolated from the reaction products obtained when atrovenetin was oxidized with alkaline potassium permanganate at room temperature.

Oxidation of atrovenetin yellow trimethyl ether, $C_{22}H_{24}O_6$, with potassium permanganate in boiling acetone gave $C_{22}H_{22}O_8$, pale-yellow needles, m.p. 306–307°, soluble in sodium bicarbonate and giving a red-brown ferric colour. The substance, which still contains three methoxyl groups, appears to be derived from the parent trimethyl ether by the oxidation of a *C*-CH_3 group to *C*-CO_2H. Oxidation of the yellow trimethyl ether at 100° with chromic acid in acetic acid gave a substance $C_{19}H_{18}O_6$, yellow-brown prisms, m.p. 277–278°, which contains only one methoxyl group and gives a crystalline oxime, $C_{19}H_{19}O_6N$, in brown-orange needles, m.p. 221°. Attempts to oxidize the trimethyl ether with selenium dioxide by protracted boiling in either acetic anhydride or pentanol led to recovery of the ether in yields of 32 and 86 % respectively, but no degradation product was isolated.

Degradation of atrovenetin by heating with electrolytic zinc dust led to the isolation in small yield (3 mg. from 3 g. of atrovenetin) of a colourless crystalline solid which gave an ultraviolet-absorption spectrum that is indistinguishable from that of pure pyrene, $C_{16}H_{10}$, and a crystalline picrate which did not depress the melting point of authentic pyrene picrate on admixture with it.

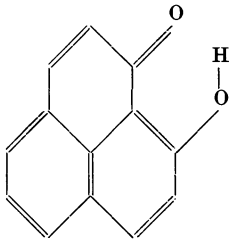
There is no doubt that from the morphological point of view *P. atrovenetum* belongs to the *P. herquei* series of species of *Penicillium* and is closely related to that species (Smith, 1956). This similarity is also evident from the chemical viewpoint. Thus, when the dried mycelium of strains of either *P. atrovenetum* or of *P. herquei* is extracted with ether or chloroform, a very dark-green extract of crude colouring matters is obtained in each case. This is particularly noteworthy since, whereas solvent extracts of a variety of colours may be obtained from different species of *Penicillium*, the production of a green ethereal or chloroform extract is surprisingly rare. Almost equally unusual is the fact that an intensely fluorescent green extract is obtained when the dried mycelium of either *P. herquei* or *P. atrovenetum* is immersed in cold concentrated sulphuric acid.

Hence it is perhaps not surprising that the empirical formulae of the pure colouring matters are so similar, i.e. atrovenetin, $C_{19}H_{18}O_6$, norherqueinone, $C_{19}H_{18}O_7$, and its methyl ether herqueinone, $C_{19}H_{17}O_6(OCH_3)$. Their physical and chemical properties are also very similar in many respects. They are all highly coloured, dextrorotatory, high-melting crystalline solids with definitely similar ultraviolet-absorption spectra. Each of them has a minimum of three and probably four methyl groups attached to carbon. They are all readily soluble in concentrated hydrochloric acid and in dilute aqueous sodium hydroxide to strongly coloured solutions, but none of them dissolves in aqueous sodium carbonate. They all give intense ferric colours in ethanol which, of course, differ in shade. Their solutions in cold concentrated sulphuric acid are yellow to reddish orange in colour with, in each case, an intense yellow-green to green fluorescence. None of them reacts with Brady's reagent. Of special significance appears to be the fact that, on methylation with dimethyl sulphate and anhydrous potassium carbonate in acetone, atrovenetin and herqueinone give, in each case, a mixture of two isomeric trimethyl ethers.

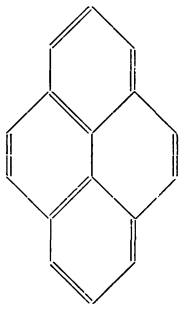
Conclusive evidence has recently been obtained (Neill & Raistrick, 1956; Barton, de Mayo, Morrison, Schaeppi & Raistrick, 1956) showing the close structural relationship of atrovenetin to the *P. herquei* colouring matters. The latter workers reduced norherqueinone, $C_{19}H_{18}O_7$, with zinc dust in acetic acid at room temperature and obtained deoxynorherqueinone, $C_{19}H_{18}O_6$, in the same way as Galarraga *et al.* (1955) obtained deoxyherqueinone, $C_{20}H_{20}O_6$, from herqueinone, $C_{20}H_{20}O_7$, and proved that deoxynorherqueinone and atrovenetin are identical and give identical triacetates.

It has been mentioned previously that, in contrast to the marked stability of atrovenetin to boiling 2N sulphuric acid, both norherqueinone and herqueinone are readily hydrolysed by this reagent, giving respectively norxanthoherquein and its monomethyl ether xanthoherquein, together with a molecule of methyl *iso*propyl ketone in each case. Barton *et al.* (1956) compared the ultraviolet-absorption spectra of xanthoherquein tetraacetate, norxanthoherquein pentaacetate, deoxyherqueinone diacetate and deoxynorherqueinone triacetate (=atrovenetin triacetate), all of which give a positive ferric reaction in ethanol, and expressed the view that all these compounds are derivatives of 9-hydroxyperinaphthen-1-one (I) (Koelsch & Anthes, 1941; Loudon & Razdan, 1954). They further postulated that the structure of atrovenetin may be represented as structure (I) with the addition of three phenolic hydroxyl groups and one CH_3

group attached to carbon, together with one ethereal oxygen probably bound up in the grouping $-CMe_2-CHMe-O-$ and attached, as a ring, to the aromatic system. The formation of pyrene (II) or a close derivative of it on zinc-dust distillation of atrovenetin is then to be explained by scission of the ethereal linkage, followed by recyclization.



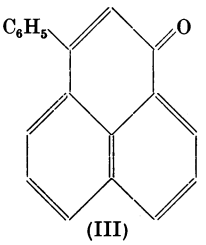
(I)



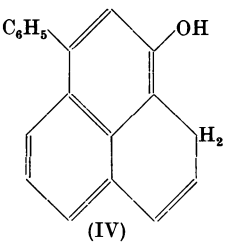
(II)

Until recently very few synthetic, and no naturally occurring, hydroxyperinaphthenones had been described. However, Cooke & Segal (1955*a*) reported a unique red crystalline glycoside, haemocorin, which they obtained from the bulbous roots of the Australian plant, *Haemodorum corymbosum* Vahl. Haemocorin is readily hydrolysed with dilute acid to cellobiose and a purple-red aglycone, $C_{20}H_{14}O_4$, which contains one methoxyl group and gives a diacetate and isomeric mono- and di-methyl ethers. From a study of the functional derivatives, degradation products and absorption spectra of the aglycone, Cooke & Segal (1955*b*) concluded that the aglycone is a dihydroxymethoxyphenylperinaphthenone and suggest that it is probably either 2:6-dihydroxy-5-methoxy-7-phenyl- or 4:9-dihydroxy-3-methoxy-5-phenyl- or 2:4-dihydroxy-5-methoxy-7-phenyl-perinaphthen-1-one. Comparison of the properties of the haemocorin aglycone, atrovenetin, xanthoherquein and norxanthoherquein reveals some noteworthy resemblances. Thus all these compounds, in common with 9-hydroxyperinaphthen-1-one, show a hydrogen-bonded and conjugated carbonyl group in the infrared between 1620^{-1} and 1630^{-1} cm. Similarly, the haemocorin aglycone gives two pairs of isomeric methyl ethers, i.e. monomethyl ethers A and B and dimethyl ethers A and B; atrovenetin gives three pairs, i.e. monomethyl ethers A and B, trimethyl ethers A and B and tetramethyl ethers A and B. Cooke & Segal (1955*b*) synthesized 4-hydroxyperinaphthen-1-one and 6-hydroxy-4-phenylperinaphthen-1-one and showed that each of these compounds gave two isomeric methyl ethers. Finally the dimethyl ether A of haemocorin aglycone and atrovenetin tetramethyl ether A are readily partially demethylated by boiling with very dilute ethanolic mineral acid, i.e. sulphuric or hydrochloric acid, giving in the latter case atrovenetin trimethyl ether.

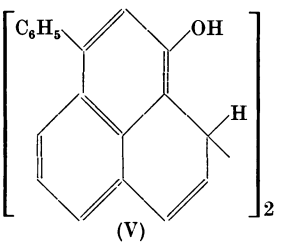
Koelsch & Anthes (1941) showed that the yellow 3-phenylperinaphthen-1-one (structure III) is readily reduced with zinc dust and acetic acid in an atmosphere of hydrogen to give a colourless product of structure (IV) which is readily oxidized in air. A ligroin solution of (III) reacts with an equivalent amount of (IV) in the same solvent to give a quantitative yield of the quinhydrone (V) which is deep red in colour. Since many examples are known among fungal metabolic products of the simultaneous occurrence in fungal cultures of the quinonoid and quinol forms of such products, and even of the quinhydrone in some cases, we believe that the intense green colour of extracts of the mycelium of *P. atrovenetum* and of *P. herquei* may well be due to the quinhydrones of structures, analogous to (V), of atrovenetin on the one hand and of herqueinone or norherqueinone on the other hand.



(III)



(IV)



(V)

EXPERIMENTAL

Cultures

Atrovenetin has been isolated from each of the following strains of *Penicillium atrovenetum* G. Smith:

(*a*) The type strain (L.S.H.T.M. Cat. no. S.M. 683) was isolated here in May 1952 by Mrs S. Marcus from soil from the Sussex Downs.

(*b*) Strain S.M. 715 was isolated here in June 1952 by Mrs S. Marcus from soil from the sand dunes at Perranporth, Cornwall.

(*c*) Strain M. 467 was isolated here in 1943 by Mrs J. M. Webb from the soil of a mangold field in Norfolk.

A detailed description of the species is given by Smith (1956).

Cultural conditions and characteristics

Czapek–Dox solution (glucose, 50·0 g.; $NaNO_3$, 2·0 g.; KH_2PO_4, 1·0 g.; KCl, 0·5 g.; $MgSO_4,7H_2O$, 0·5 g.; $FeSO_4$, $7H_2O$, 0·01 g.; water, 1000 ml.) was distributed in 350 ml.

amounts in 1 l. conical flasks. The flasks were plugged with cotton wool and sterilized. The contents of the flasks were then inoculated with a spore suspension in water prepared from Czapek–agar slopes of the strain of *P. atrovenetum* under investigation, three flasks being sown from each slope. The flasks were incubated in the dark at 24°.

During the incubation period the mycelium of strain S.M. 683, which was used for the bulk preparation of atrovenetin, became very folded. The upper surface, which after 7 days incubation was a bright bluish green colour, became much darker after 21 days and showed a few yellowish green pitted areas. The reverse surface, initially brownish orange in colour, gradually darkened to olive brown. The culture fluid was lemon yellow in colour after 7 days, becoming brownish orange after 21 days. Cultures of strains S.M. 715 and M. 467 were very similar in appearance to those of strain S.M. 683.

At intervals during the incubation period portions of the mycelium of strain S.M. 683 were pressed as dry as possible between filter papers. Treatment of some of this material with much cold conc. H_2SO_4 gave an immediate lemon-yellow solution with a strong green fluorescence after 7 days, increasing in intensity after 14 and 21 days incubation. Extraction of other portions of the dried mycelium with ethanol gave highly coloured solutions increasing from orange brown to very intense dark brown with age and giving most striking ferric reactions, increasing from dark brown after 7 days to almost black after 21 days incubation. The mycelium of strains S.M. 715 and M. 467 gave similar but less intense reactions.

A number of cultural details of representative batches of the three strains of *P. atrovenetum*, including yields of atrovenetin, are collected in Table 1. The five batches of strain S.M. 683 are representative of twelve batches harvested over a period of 2½ years and are arranged in chronological order.

Isolation of the mycelial colouring matters

At the end of the incubation period the mycelium from each strain was separated, by straining through muslin, from the culture fluid, which was discarded after measuring its pH and glucose content by polarimeter. The mycelium was washed with water, squeezed as dry as possible in a tincture press and dried in a vacuum oven at 40–45°. The dried mycelium (Table 1, column 6) was finely ground in a coffee mill and extracted for two working days in a large copper Soxhlet with light petroleum (b.p. 40–60°). The extract, on concentration, gave a brown oil (Table 1, column 7). The extracted mycelium was re-dried and was then re-extracted in the Soxhlet with anaesthetic ether for 8 days when the extract, which was initially very dark green in colour, had become almost colourless. The solid crude colouring matter which separated was collected each day and dried. The total combined crude colouring matter (Table 1, column 8) was finely ground and intimately mixed with about an equal volume of clean sand and the mixture was Soxhlet-extracted with acetone.

The yellow-brown colouring matter which separated from the acetone extracts from *P. atrovenetum* strain S.M. 683 consisted of crystalline atrovenetin. It was collected frequently and separated into two main fractions: (*a*) m.p. 288° (decomp.) or above which was usually pure enough for the preparation of derivatives, and (*b*) m.p. usually between 260° and 270° (decomp.). The weights and m.p.'s of these fractions are given in Table 1, columns 9 and 10. Fraction (*b*) was further purified by crystallization from dioxan, from which solvent atrovenetin separated with dioxan of crystallization which was removed by immersing the crystals in a little cold acetone, when they slowly effloresced to yield solvent-free atrovenetin, m.p. 285° and above.

Strain M. 467 gave a good yield of crude atrovenetin, 6·25 g. from thirty-five flasks, which although of low m.p., 263° (decomp.), had almost the same optical rotation as pure atrovenetin from strain S.M. 683, i.e. $[\alpha]^{22}_{5461}+150°$, $[\alpha]^{22}_{5790}+116°$, in dioxan (*c*, 1·000). Its identity was confirmed by conversion into the perchlorate, orange-yellow needles, m.p. 230° (decomp.) (see p. 171); the hydrochloride, yellow needles, m.p. 288° (decomp.) (see p. 171); and the two isomeric methyl ethers by methylation with diazomethane, i.e. the yellow trimethyl ether, m.p. 169° and mixed m.p. 168–169°, with material prepared from strain S.M. 683, and the orange trimethyl ether, m.p. 177–179° and mixed m.p. 178–179° (see p. 172).

The ether extract from strain S.M. 715 contained a much smaller proportion of atrovenetin than strains S.M. 683 or M. 467. The ether extract (10·35 g.) consisted of a very

Table 1. *Cultural details and yields of crude atrovenetin from strains of* Penicillium atrovenetum *G. Smith*

Strain no.	Incubation period (days)	No. of flask cultures	Final pH of culture medium	Residual glucose by polarimeter (%)	Wt. of dried mycelium (g.)	Light petroleum extract 'fat' (g.)	Ether extract (g.)	Yields of crystalline atrovenetin	
								g.	M.p.
S.M. 683	28	32	4·5	0·25	129	3·7	19·02	8·8	280°
S.M. 683	21	100	4·6	0·39	396	22·2	45·7	4·3	292
								5·0	290
								10·2	260
S.M. 683	21	150	4·6	0·68	573	27·1	90·4	25·8	288
								15·8	285
S.M. 683	21	149	4·0	0·61	532	—	76·6	2·95	292
								36·2	263
								5·1	257
S.M. 683	21	148	4·0	0·55	542	16·3	84·0	22·0	290
S.M. 715	21	50	4·9	0·36	190	15·2	10·35	0·70	260
M. 467	28	35	4·0	1·31	91	—	—	6·25	263

dark-green solid from which only 0·7 g. of crude atrovenetin was obtained after trituration with acetone. Portions of this material were converted into the perchlorate, 0·1 g. giving 0·103 g. of perchlorate, m.p. 228° (decomp.) and not depressed on admixture with atrovenetin perchlorate, m.p. 230° (decomp.) from strain S.M. 683. The presence of atrovenetin was confirmed by methylating a second portion with diazomethane, which gave the yellow trimethyl ether of atrovenetin, m.p. 162–163° (see p. 172), not depressed on admixture with a specimen, m.p. 168–169° from strain S.M. 683.

Purification and general properties of atrovenetin

Atrovenetin was purified for analysis either by crystallization from acetone as yellow-brown rectangular plates, or from dioxan as yellow-brown prisms containing solvent of crystallization, or by sublimation in high vacuum at 210° as yellow-orange rectangular plates, the m.p. of all three specimens being 295° (decomp.). (Found, on specimen from strain S.M. 683: C, 66·8; H, 5·3; N, S, Cl, nil; *C*-CH_3, 8·4, 9·5; OCH_3, nil; on specimen from strain M. 467: C, 66·8; H, 5·6. $C_{19}H_{18}O_6$ requires C, 66·7; H, 5·3; 2*C*-CH_3, 8·8%.) $[\alpha]^{21}_{5461}$ +154°, $[\alpha]^{21}_{5790}$ +116° in dioxan (*c*, 0·486). Owing to the relatively slight solubility of atrovenetin and its derivatives in organic solvents and the intense colour of the solutions obtained, the determination of their optical rotations is difficult and is subject to a large error.

Atrovenetin is not readily soluble in the usual organic solvents but dissolves in hot acetone, acetic acid and dioxan. Its golden solution in dioxan has an intense yellow-green fluorescence. It dissolves in cold aqueous 2N-NaOH to give an orange solution which changes reversibly on warming to a deep red colour. It does not dissolve in aqueous Na_2CO_3. It gives a yellow solution in cold conc. H_2SO_4 with an intense yellow-green fluorescence. Its ethanolic solution gives an intense red-brown colour with ethanolic $FeCl_3$. It does not react with Brady's reagent for carbonyl groups (0·32% 2:4-dinitrophenylhydrazine in aqueous 2N-HCl), nor does it give any colour on reduction with magnesium and HCl or with sodium amalgam followed by acidification. It slowly gives a blue colour with 2:6-dibromoquinoneimide (Gibbs, 1927).

Atrovenetin readily gives a water-stable boroacetate as follows: a mixture of atrovenetin (0·2 g.), boroacetic anhydride (0·3 g.) (Dimroth & Faust, 1921; Dimroth, 1926) and acetic anhydride (1·5 ml.) was refluxed for 10 min. On cooling, the bright orange-yellow solid which separated was collected, washed well with ether and dried. It did not melt below 360° and was recovered unchanged after refluxing with water for 1 hr.

Functional derivatives of atrovenetin

Atrovenetin hydrochloride. Atrovenetin (0·2 g.) was suspended in warm acetic acid (20 ml.). Conc. HCl (2 ml.) was added to the mixture, when atrovenetin immediately dissolved to give an orange-yellow solution from which *atrovenetin hydrochloride* crystallized on cooling and was recrystallized from the same solvent mixture as yellow needles (0·23 g.), m.p. 285–286° (decomp.). (Found, on sample dried in high vacuum at room temp.: C, 60·3; H, 5·2; Cl, 9·5. $C_{19}H_{18}O_6$.HCl requires C, 60·2; H, 5·1; Cl, 9·4%.) The hydrochloride decomposes on heating at 220° in high vacuum giving a chlorine-free sublimate of atrovenetin, m.p. 292°.

Atrovenetin perchlorate. Perchloric acid (1 ml. of 60% (w/v)) was added to a suspension of atrovenetin (0·2 g.) in hot acetic acid (5 ml.). The pigment dissolved and, on cooling, yellow needles (0·15 g.), m.p. 225° (decomp.) separated and were collected and crystallized from ethyl acetate giving *atrovenetin perchlorate dihydrate* as yellow needles, m.p. 230° (decomp.). (Found: C, 47·3; H, 4·9; Cl, 7·3. $C_{19}H_{18}O_6.HClO_4,2H_2O$ ($=C_{19}H_{23}O_{12}Cl$) requires C, 47·65; H, 4·8; Cl, 7·4%.)

Triacetylatrovenetin. A mixture of atrovenetin (0·5 g.), acetic anhydride (5 ml.) and anhydrous sodium acetate (0·1 g.) was heated on the boiling-water bath for 2 hr. The orange-brown solid obtained when the acetylation mixture was poured into ice–water (200 ml.) was collected, washed with water and dried. The dried solid was extracted with cold ether. The ether extract was concentrated, giving a crystalline solid on standing, m.p. 183–186°, recrystallization of which from methanol to constant m.p. gave *triacetylatrovenetin* as orange-brown cubes (0·14 g.), m.p. 187–188°. The yellow methanolic solution of the acetate has an intense yellow-green fluorescence and gives a red-brown ferric reaction. (Found: C, 63·8; H, 5·3; CH_3.CO, 28·3. $C_{25}H_{24}O_9$ requires C, 64·1; H, 5·2; 3CH_3.CO, 27·6%.) $[\alpha]^{22}_{5461}$ +104°, $[\alpha]^{22}_{5790}$ +84° in acetic acid (*c*, 1·00). Acetylation of atrovenetin with acetic anhydride and $HClO_4$ as catalyst at room temp., or with sodium acetate or pyridine as catalyst under reflux, led to products which could not be crystallized.

Atrovenetin monomethyl ethers A and B

Atrovenetin monomethyl ether A. A solution of diazomethane in ether prepared from nitrosomethylurea (10 g.) was added to a suspension of atrovenetin (5 g.) in methanol (30 ml.) and ether. The pigment quickly dissolved and when the initially vigorous reaction had moderated (about 3 min.) an orange crystalline solid separated (1·4 g.), m.p. 248–250° and was collected by filtration. (For treatment of the filtrate see p. 172.) The substance was recrystallized from a large volume of acetone giving *atrovenetin monomethyl ether A* (1·27 g.) as orange-red rectangular plates, m.p. 262–263°. (Found: C, 67·4, 67·4; H, 5·65, 5·74; N, nil; OCH_3, 9·0; *C*-CH_3, 9·2. $C_{20}H_{20}O_6$ requires C, 67·4; H, 5·7; 1 OCH_3, 8·7; 2*C*-CH_3, 8·4%.) $[\alpha]^{21}_{5461}$ +224°, $[\alpha]^{21}_{5790}$ +176° in $CHCl_3$ (*c*, 0·504). The ether is soluble in aqueous 2N-NaOH to an orange solution but does not dissolve in aqueous Na_2CO_3. It dissolves in cold conc. H_2SO_4 to a yellow solution with an intense yellow-green fluorescence. Its ethanolic solution gives an intense red-brown ferric reaction.

Diacetylatrovenetin monomethyl ether A. A mixture of atrovenetin monomethyl ether A (0·3 g.), acetic anhydride (5 ml.) and anhydrous sodium acetate (0·2 g.) was heated at 100° for 8 hr. The acetic anhydride was removed under reduced pressure and the residue was crystallized from methanol giving *diacetylatrovenetin monomethyl ether A* as glistening golden needles (0·135 g.), m.p. 218–219°. (Found: C, 65·5; H, 5·6; OCH_3, 6·8; CH_3.CO, 20·5. $C_{24}H_{24}O_8$ requires C, 65·45; H, 5·5; 1 OCH_3, 7·0; 2CH_3.CO, 19·5%.) $[\alpha]^{22}_{5461}$ +105°, $[\alpha]^{22}_{5790}$ +101° in dioxan (*c*, 0·501). The substance is not soluble in aqueous 2N-NaOH. Its methanolic solution has an intense yellow-green fluorescence and gives an intense red-brown ferric reaction.

Diethyl ether of atrovenetin monomethyl ether A. A mixture of atrovenetin monomethyl ether A (0·5 g.) dry acetone (50 ml.), anhydrous K_2CO_3 (2 g.) and diethyl sulphate (0·6 ml.) was refluxed for 3 hr. Additional diethyl sulphate (0·4 ml.) and K_2CO_3 (1 g.) were then added and refluxing was continued for a total of 8 hr. The ethylation product (0·27 g.) was worked up in the usual way and purified by crystallization from methanol, giving the *diethyl ether of atrovenetin monomethyl ether A* as yellow needles, m.p. 129–130°. The substance is not soluble in aqueous 2 N-NaOH but its ethanolic solution gives an intense red-brown ferric reaction. [Found: C, 69·9; H, 6·9; alkoxyl as OCH_3, 22·8. $C_{24}H_{28}O_8$ ($=C_{19}H_{18}O_6+CH_2+2C_2H_4$) requires C, 69·9; H, 6·8; three alkoxyl groups calculated as 3 OCH_3, 22·6%.] $[\alpha]_{5461}^{21}+128°$, $[\alpha]_{5790}^{21}+104°$ in ethanol (*c*, 0·497).

Atrovenetin monomethyl ether B. A mixture of atrovenetin (0·3 g.) in aqueous 20% NaOH (20 ml.) and dimethyl sulphate was shaken in the cold, the reaction being maintained alkaline. A pale-yellow crystalline sodium salt, m.p. 340–341° (decomp.) separated and was collected (0·11 g.). It was suspended in dilute HCl, extracted with ether and the extract was evaporated to dryness. The residue was crystallized from aqueous methanol giving *atrovenetin monomethyl ether B* as yellow plates, m.p. 226–227°. (Found: C, 67·1; H, 5·7; OCH_3, 8·3. $C_{20}H_{20}O_6$ requires C, 67·4; H, 5·7; 1 OCH_3, 8·7%.) This ether is soluble in $NaHCO_3$ to a yellow solution, and in cold conc. H_2SO_4 to a yellow solution with an intense yellow-green fluorescence. It gives a green ferric reaction in ethanol and a negative reaction with Brady's reagent.

Atrovenetin trimethyl ethers—yellow and orange

(*a*) *Yellow trimethyl ether.* The ethereal diazomethane filtrate from the preparation of atrovenetin monomethyl ether A was kept overnight at 0° and the excess of diazomethane was destroyed with acetic acid. The ether solution was then washed successively with aqueous N-NaOH, N-HCl and water, dried ($MgSO_4$) and evaporated to dryness. The crystalline residue was washed with a little methanol to remove gum. This residue (2·26 g.) was repeatedly crystallized from methanol giving *yellow atrovenetin trimethyl ether* as bright-yellow rectangular plates, m.p. 168–169°. (Found: C, 68·4; H, 6·4; OCH_3, 24·25; *C*-CH_3, 11·8; mol.wt., cryoscopic in camphor, 361. $C_{22}H_{24}O_6$ requires C, 68·7; H, 6·3; 3 OCH_3, 24·2; 3*C*-CH_3, 11·7%; mol.wt. 384.) $[\alpha]_{5461}^{21}+124°$, $[\alpha]_{5790}^{21}+122°$ in methanol–$CHCl_3$ (*c*, 1·036).

The yellow methyl ether is soluble in ether, $CHCl_3$, acetone, and in hot methanol or ethanol, the ethanolic solution having a yellow-green fluorescence and giving an intense red-brown ferric reaction. The ether is readily soluble in conc. HCl giving a yellow solution, and its solution in cold conc. H_2SO_4 has an intense yellow-green fluorescence. It is not soluble in aqueous 2 N-NaOH but when sodium methoxide is added to a methanolic solution of the ether the sparingly soluble sodium salt separates as pale-yellow plates, m.p. 344° (decomp.). (Found: C, 64·5; H, 5·5; Na, 5·7. $C_{22}H_{23}O_6Na$ requires C, 65·0; H, 5·7; Na, 5·7%.) The methyl ether does not react with 2:4-dinitrophenylhydrazine nor does it give any colour on reduction with magnesium and HCl or with sodium amalgam followed by acidification. It may also be prepared, though less readily, by methylating atrovenetin with dimethyl sulphate or methyl iodide and K_2CO_3 in acetone.

Ferrichloride of yellow trimethyl ether. A solution of atrovenetin yellow trimethyl ether (0·1 g.) in conc. HCl (1 ml.) was mixed with a solution of $FeCl_3$ (0·2 g.) in conc. HCl (2 ml.). The resulting orange precipitate was collected, dried and crystallized from acetic acid containing $FeCl_3$ and conc. HCl. The *ferrichloride* (88 mg.) forms deep-red orange rhombs, m.p. 148° (decomp.). (Found: C, 45·6; H, 4·5; Fe, 9·3; OCH_3, 15·85. $C_{22}H_{24}O_6.HFeCl_4$ requires C, 45·3; H, 4·3; Fe, 9·6; 3 OCH_3, 16·0%.)

Acetate perchlorate of yellow trimethyl ether. Perchloric acid (60%; 12 drops) was added to a suspension of the yellow trimethyl ether (0·5 g.) in acetic anhydride (3 ml.). The mixture became warm and the ether dissolved. The solution was heated at 100° for 5 min. On cooling, the orange solid which slowly separated was collected, dried and crystallized from methanol giving the *acetate perchlorate* (0·46 g.) as orange-yellow plates, m.p. 185° (decomp.), the m.p. varying with the rate of heating. (Found: C, 54·3; H, 5·3; Cl, 6·55; OCH_3, 17·7. $C_{24}H_{26}O_7.HClO_4$ requires C, 54·7; H, 5·2; Cl, 6·7; 3 OCH_3, 17·7%.) $[\alpha]_{5461}^{22}+148°$, $[\alpha]_{5790}^{22}+132°$ in acetic acid (*c*, 0·500). An ethanolic solution of this compound has an intense yellow-green fluorescence but gives no ferric reaction. All attempts failed to acetylate the yellow trimethyl ether with acetic anhydride and anhydrous sodium acetate, or pyridine, or conc. H_2SO_4, as catalyst, the starting material being the only crystalline product recovered.

(*b*) *Orange trimethyl ether.* This ether has been prepared in three ways.

(i) The methanol mother liquors and washings from the methylation of atrovenetin with diazomethane were combined and evaporated to dryness. The residue was fractionally crystallized from methanol and was separated by hand sorting into further amounts of the yellow trimethyl ether together with a small amount of an orange isomer. This was recrystallized from methanol giving the *orange trimethyl ether* (0·28 g.) as orange rectangular plates, m.p. 178–179°, mixed m.p. with the yellow trimethyl ether 139–142°. (Found: C, 68·55; H, 6·25; OCH_3, 24·2. $C_{22}H_{24}O_6$ requires C, 68·7; H, 6·3; 3 OCH_3, 24·2%.) $[\alpha]_{5461}^{22}+138°$, $[\alpha]_{5790}^{22}+112°$ in ethanol (*c*, 0·505).

(ii) Atrovenetin monomethyl ether A (0·1 g.), m.p. 262–263°, suspended in methanol, was methylated for 36 hr. at 0° with excess of ethereal diazomethane. The ether solution was then washed successively with 2 N-NaOH, N-HCl and water, dried ($MgSO_4$) and evaporated to dryness. The residue was crystallized from methanol giving orange rectangular plates (0·04 g.), m.p. and mixed m.p. with the orange trimethyl ether prepared as in (i) above, 178–179°.

(iii) A mixture of atrovenetin monomethyl ether A (2 g.), dry acetone (600 ml.), dimethyl sulphate (1·17 ml., 2·2 moles) and anhydrous K_2CO_3 (2 g.) was refluxed for 3 hr. Additional K_2CO_3 (2 g.) and dimethyl sulphate (0·9 ml.) were added and heating was continued for a further 3 hr. The methylation product was worked up in the usual way and crystallized from methanol giving the orange trimethyl ether (1·73 g.), m.p. and mixed m.p. 178–179°.

The orange trimethyl ether is soluble in ether, $CHCl_3$, acetone and in hot methanol and ethanol. It does not dissolve in aqueous 2 N-NaOH but gives an intense red-brown ferric reaction in ethanol. It could not be acetylated with sodium acetate and acetic anhydride.

Ferrichloride of orange trimethyl ether. This derivative was prepared and crystallized in the same way as the ferri-

chloride of the yellow trimethyl ether; yield 0·14 g. from 0·1 g. of ether. It crystallizes in deep-red orange rhombs, m.p. 151–152°; mixed m.p. with the ferrichloride of the yellow trimethyl ether, 125–130°. (Found: C, 45·3; H, 4·4; Fe, 9·25; OCH_3, 15·8. $C_{22}H_{24}O_6.HFeCl_4$ requires C, 45·3; H, 4·3; Fe, 9·6; 3 OCH_3, 16·0%.)

Atrovenetin tetramethyl ethers A and B

A (i). From atrovenetin yellow trimethyl ether. A mixture of the yellow trimethyl ether (1 g.), sodium-dried benzene (120 ml.), methyl iodide (20 ml.) and freshly precipitated and dried Ag_2O (2 g.) was heated under reflux. Further Ag_2O was added at 2-hourly intervals, i.e. 4 × 1 g., followed by 4 × 0·5 g., and refluxing was continued for a total of 24 hr. Solvents were then removed by evaporation and the residue was extracted with ether. The ether extract, evaporated to 20 ml., was chromatographed on Al_2O_3 (2·5 cm. × 14 cm.) which was then eluted with ether in 100 ml. fractions. The first and second fractions (200 ml.) of eluate were combined, the solvent was removed and the residue was washed with light petroleum (b.p. 40–60°). The residual yellow crystalline solid (0·29 g.), m.p. 131–134°, was crystallized from aqueous methanol giving *atrovenetin tetramethyl ether A* (0·21 g.) as yellow needles, m.p. 141–143°. (Found: C, 69·2; H, 6·8; OCH_3, 30·0. $C_{23}H_{26}O_6$ requires C, 69·3; H, 6·6; 4 OCH_3, 31·15%.) $[\alpha]_{5461}^{21}$ + 156°, $[\alpha]_{5790}^{21}$ + 126° in ethanol (*c*, 1·000).

A (ii). From atrovenetin orange trimethyl ether. The orange trimethyl ether was methylated and the product was purified in the same way as is described in section (i) immediately above; yield 0·14 g. from 0·5 g. of trimethyl ether. Yellow needles, m.p. 139–140°; mixed m.p. 140–141° with the tetramethyl ether A (m.p. 141–143°) described above. (Found: C, 69·4; H, 6·7; OCH_3, 30·2. $C_{23}H_{26}O_6$ requires C, 69·3; H, 6·6; 4 OCH_3, 31·15%.) $[\alpha]_{5461}^{21}$ + 146°, $[\alpha]_{5790}^{21}$ + 122° in ethanol (*c*, 0·994).

Identical ultraviolet- and infrared-absorption spectra were given by the two specimens of atrovenetin tetramethyl ether A described in sections (i) and (ii) above.

Atrovenetin tetramethyl ether A does not dissolve in aqueous 2 N-NaOH and gives no ferric reaction in ethanolic solution. Its yellow solutions in cold conc. H_2SO_4 and in ethanol have an intense yellow-green fluorescence. It is readily soluble in most organic solvents except light petroleum.

Atrovenetin tetramethyl ether A was readily partially demethylated to atrovenetin orange trimethyl ether as follows. A solution of the ether (60 mg.) in ethanol (10 ml.) containing conc. HCl (0·3 ml.) was refluxed for 4 hr. Ethanol was then removed by evaporation and the residue was neutralized with aqueous $NaHCO_3$ and extracted with ether. The dried (Na_2SO_4) extract was evaporated and the crystalline residue (43 mg.), m.p. 173–175°, was crystallized from methanol giving atrovenetin orange trimethyl ether as orange rectangular plates (25 mg.), m.p. 174–175°, mixed m.p. with an authentic specimen 176–178°. The product also gave an intense red-brown ferric reaction in ethanol.

B. From atrovenetin yellow trimethyl ether. The third and fourth fractions (200 ml.) of ether eluate from section (A i) above were combined and evaporated, and the residue was crystallized from light petroleum (b.p. 40–60°) as yellow cubes (41 mg.), m.p. 181–182°. Recrystallization from aqueous methanol gave *atrovenetin tetramethyl ether B* as yellow prisms, m.p. 186–187°. (Found: C, 69·3; H, 6·6; OCH_3, 30·7. $C_{23}H_{26}O_6$ requires C, 69·3; H, 6·6; 4 OCH_3, 31·15%.) This ether is insoluble in aqueous 2 N-NaOH and gives a definite brown or red-brown ferric reaction in ethanol which is discharged on the addition of water. Its yellow solution in cold conc. H_2SO_4 has a yellow fluorescence.

Degradation experiments with atrovenetin and its derivatives

A. Acid hydrolysis experiments. A mixture of atrovenetin [1 g., m.p. 295° (decomp.)] in aqueous 2 N-H_2SO_4 (150 ml.) was refluxed for 11 hr. in a slow stream of O_2- and CO_2-free Na_2. The issuing N_2 was passed first through bubblers containing Brady's reagent and then through standard $Ba(OH)_2$ solution. No precipitate was formed in the Brady solution and only traces of $BaCO_3$ were precipitated. The mixture was then distilled to about 30 ml., water (100 ml.) was added and the distillation was repeated. The combined distillates, neutral in reaction, gave no precipitate with Brady's reagent. The solid in the H_2SO_4 suspension was collected, washed with water and dried, wt. 0·976 g., m.p. 285° (decomp.). It was shown to be substantially unchanged atrovenetin by methylation in ether–methanol with ethereal diazomethane (see p. 171), when atrovenetin monomethyl ether A (36 mg.), m.p. and mixed m.p. 256°, atrovenetin yellow trimethyl ether (0·32 g.), m.p. and mixed m.p. 164–165°, and atrovenetin orange trimethyl ether (54 mg.), m.p. and mixed m.p. 179–180°, were obtained.

Atrovenetin was also recovered unchanged (0·42 g.), m.p. and mixed m.p. 289° (decomp.), after a solution of it (0·5 g.) in methanol (50 ml.) containing conc. H_2SO_4 (0·75 g.) was boiled for 6 hr.

B. Alkaline hydrolysis experiments. A solution of atrovenetin (1 g.) in aqueous N-KOH (100 ml.) was refluxed for 48 hr. in a slow stream of purified N_2. The effluent N_2 was passed through bubblers containing standard $Ba(OH)_2$ solution. The KOH solution was cooled and acidified through a tap funnel with 4 N-H_2SO_4, the stream of N_2 being continued. Titration of the $Ba(OH)_2$ solution showed that only traces of CO_2 had been formed. The orange solid precipitated in the acidified KOH was collected (0·952 g.) and a portion of it was methylated with diazomethane giving atrovenetin yellow trimethyl ether as yellow rectangular plates, m.p. and mixed m.p. 168–169°.

The yellow and orange trimethyl ethers of atrovenetin were recovered unchanged in 68–92% yield after refluxing for 3–8 hr. either in 10% methanolic KOH or in 10% methanolic HCl.

C. Potash-fusion experiments. Atrovenetin (1 g.) was added in portions to a stirred melt of KOH (10 g.) and water (1 ml.) at 210°. The temp. was then slowly raised to 300° and held there for 15 min. The cooled melt was dissolved in water (100 ml.) and acidified with H_2SO_4. The resulting orange precipitate was collected and extracted with ether. Evaporation of the ether extract gave an orange-brown crystalline solid (0·6 g.), m.p. 292° (decomp.), which gave the usual colour reactions of atrovenetin, sublimed in high vacuum at 210° in yellow-brown rectangular plates, m.p. 293° (decomp.) and gave atrovenetin yellow trimethyl ether, m.p. and mixed m.p. 168–169°, on methylation with diazomethane.

Variations in the temp. of the melt, held for 2 hr. in each case, led to the following results: 325–335°, 0·563 g. of recovered pigment, m.p. 292–294°; 330–340°, 0·16 g. of

pigment, m.p. 284–286° (decomp.); 340–350°, complete decomposition. The acid filtrates from all these experiments were combined and continuously extracted with ether. The only recognizable breakdown product isolated from the ether extract was oxalic acid (82 mg.).

Fusion of atrovenetin yellow trimethyl ether (0·5 g.) with KOH (10 g.) and water (1 ml.) for 15 min. at 280–300° yielded only resinous products.

D. Reduction experiments. (i) $LiAlH_4$ *reduction of the atrovenetin trimethyl ethers.* (*a*) *Atrovenetin yellow trimethyl ether.* A percolator containing this trimethyl ether (3 g.) was attached to a reflux condenser and the whole was connected with a reaction flask containing sodium-dried ether (approx. 450 ml.) and a slurry of $LiAlH_4$ (1·5 g.). The mixture was refluxed for 0·5 hr. after all the trimethyl ether had been extracted. Excess hydride was decomposed with ethyl acetate, and $Al(OH)_3$ was dissolved by the addition of aqueous NaOH. The ether layer was separated, washed with water, dried and evaporated to dryness. The residue was dissolved in benzene and chromatographed on alumina.

The main *reduction product YA*, which was obtained from the later eluates, was purified by crystallization from methanol giving yellow needles (1·34 g.), m.p. 145–146°. (Found: C, 71·7, 71·6; H, 6·7, 6·5; OCH_3, 24·7. $C_{22}H_{24}O_5$ requires C, 71·7; H, 6·6; 3 OCH_3, 25·3%.) $[\alpha]_{5461}^{26}+276°$, $[\alpha]_{5790}^{26}+206°$ in ethanol (*c*, 1·000). This reduction product resists acetylation at 100° with acetic anhydride and sodium acetate.

(*b*) *Atrovenetin orange trimethyl ether.* A solution of this ether (1 g.) in dry ether (250 ml.) was added to a slurry of $LiAlH_4$ (about 0·5 g.) in dry ether, and the mixture was refluxed for 0·5 hr. The reduction product was worked up and chromatographed in benzene on Al_2O_3 as described in section (*a*) above. Eluates 3, 4 and 5 gave a product which, on crystallization from methanol, yielded *reduction product OA* as scarlet prisms (0·1 g.), m.p. 203–204°. (Found: C, 71·8, 72·0; H, 6·9, 7·1; OCH_3, 24·6. $C_{22}H_{24}O_5$ requires C, 71·7; H, 6·6; 3 OCH_3, 25·3%.) $[\alpha]_{5461}^{20}+200°$, $[\alpha]_{5790}^{20}+156°$ in dioxan (*c*, 1·000).

Neither of the reduction products gives a ferric reaction. Neither of them is soluble in aqueous 2N-NaOH, nor extractable from $CHCl_3$ solution by this reagent.

(ii) *Catalytic reduction.* A solution of atrovenetin yellow trimethyl ether (0·202 g.) in acetic acid (5 ml.) was shaken for 5 hr. with an active palladium–charcoal catalyst in an atmosphere of H_2. The total absorption of H_2 was 4·4 ml. at 18° and 760 mm. The catalyst was then separated by filtration, the filtrate evaporated to dryness *in vacuo* and the residue crystallized from methanol, giving unchanged yellow trimethyl ether (0·175 g.), m.p. and mixed m.p. 167–169°.

(iii) *With sodium in ethanol.* In parallel experiments solutions of the yellow and orange trimethyl ethers of atrovenetin (0·5 g. in each case) in ethanol (50 ml.) were refluxed, and metallic sodium (0·5 g.) was added in portions. From the reaction mixtures 0·48 g. of the yellow ether and 0·287 g. of the orange ether, recrystallized in each case from methanol, were recovered.

E. Dehydrogenation experiments. (i) A mixture of atrovenetin yellow trimethyl ether (0·5 g.), 5% palladium–charcoal catalyst (0·05 g.) and Dowtherm (10 ml.) was refluxed for 16 hr. in a slow stream of CO_2. The reaction mixture was then diluted with light petroleum (b.p. 40–60°) and filtered. The filtrate was extracted with conc. HCl and the HCl extract was diluted with much water. The resultant yellow precipitate was collected, dried and crystallized from methanol, giving unchanged atrovenetin yellow trimethyl ether (0·335 g.), m.p. and mixed m.p. 164–165°.

(ii) A mixture of atrovenetin yellow trimethyl ether (0·3 g.; 1 mole), chloranil (0·4 g.; 2 moles) and xylene (10 ml.) was refluxed for 16 hr. The cooled reaction mixture was diluted with ether, washed with aqueous 2N-NaOH, dried (Na_2SO_4) and evaporated. The crystalline residue was washed with a little cold methanol and collected; wt. 0·19 g., m.p. 163–165° undepressed on admixture with atrovenetin yellow trimethyl ether.

F. Oxidation experiments on atrovenetin. (i) *With alkaline* H_2O_2. Hydrogen peroxide (30%; 20 ml.) was added slowly to a solution of atrovenetin (1 g.) in aqueous 2N-NaOH (80 ml.). The mixture became green, and after 2 hr. further H_2O_2 (20 ml.) and 10N-NaOH (10 ml.) were added. The mixture was kept overnight at room temp., filtered and the pale straw-coloured filtrate was acidified. The resulting colourless crystalline precipitate (64 mg.), m.p. 176°, was collected, dried and crystallized from benzene–light petroleum (b.p. 60–80°), giving *oxidation product A* as colourless needles, m.p. 188°. The substance gave a faint-brown ferric reaction and did not give a precipitate with Brady's reagent. (Found, on material dried *in vacuo* at 100°: C, 58·85; H, 5·23; equivalent by titration, 156. $C_{15}H_{16}O_7$ requires C, 58·43; H, 5·23%; equiv. (dibasic), 154·1.)

The acid filtrate from oxidation product A was extracted with ether, and the ether solution was extracted with $NaHCO_3$. Acidification and ether extraction of the $NaHCO_3$ solution gave a colourless, partly crystalline gum (0·46 g.) which gave a wine-red ferric colour but no reaction with Brady's reagent. The gum was sublimed in high vacuum at 130–150° and the sublimate of colourless rosettes was purified by crystallization from ether–light petroleum (b.p. 60–80°) and resublimation giving *oxidation product B* (51 mg.) as colourless rosettes of needles, m.p. 151–152°. The product gave a red-brown ferric reaction. (Found: C, 54·4; H, 2·86; equiv. by titration, 90·4. $C_{12}H_8O_7$ requires C, 54·6; H, 3·05%; equiv. (tribasic), 88·1.)

(ii) *With conc.* HNO_3. A mixture of atrovenetin (1 g.) and conc. HNO_3 (5 ml.) was heated at 100° until the evolution of nitrous fumes ceased. It was then cooled, diluted with water and extracted with ether. The ether solution was extracted with aqueous $NaHCO_3$, which was acidified, and the precipitated solid was collected and crystallized from ethyl acetate–light petroleum (b.p. 60–80°); wt. 163 mg., m.p. 234–235°. The *oxidation product* was further crystallized thrice from water containing a few drops of HCl, giving cream rectangular plates, m.p. 239–240°; $[\alpha]_{5461}^{22}+376°$, $[\alpha]_{5790}^{22}+332°$ in acetic acid (*c*, 1·00). (Found, on different samples: C, 46·9, 46·9; H, 4·23, 4·20; N, 6·3; *C*-CH_3, 9·9. $C_{15}H_{16}O_{10}N_2$, i.e. $C_{15}H_{14}O_9N_2+H_2O$, requires C, 46·9; H, 4·20; N, 7·3; 3*C*-CH_3, 11·7%.) The substance does not give a positive fluorescein test. It gives no colour with $FeCl_3$ in ethanol.

The oxidation product (180 mg.) was dissolved in a methanol–ether mixture, and an ether solution of diazomethane was added to it. A colourless solid separated immediately, and after 5 min. the excess of diazomethane was destroyed and the solid was collected, wt. 123 mg., m.p. 250–252°. Recrystallization from ethyl acetate gave the *methyl derivative of the oxidation product* as cream rectangular plates, m.p. 251°. It is only slowly soluble in

dilute aqueous NaOH and gives no colour with $FeCl_3$; $[\alpha]_{5461}^{21} + 226°$, $[\alpha]_{5790}^{21} + 204°$ in dioxan (*c*, 1·00). (Found: C, 50·6; H, 4·38; N, 6·6; OCH_3, 7·95. $C_{16}H_{16}O_9N_2$, i.e. $C_{15}H_{14}O_9N_2 + CH_2$, requires C, 50·5; H, 4·24; N, 7·4; 1 OCH_3, 8·2 %.)

G. Oxidation experiments on atrovenetin yellow trimethyl ether. (i) *With* $KMnO_4$. A boiling solution of atrovenetin yellow trimethyl ether (1 g.) in acetone (30 ml.) was oxidized with $KMnO_4$ in acetone until the oxidation proceeded slowly. The mixture was then evaporated to dryness and the residue was suspended in water and saturated with SO_2, giving a yellow-orange precipitate which was collected by filtration. The filtrate was extracted with ether from which only oxalic acid was recovered. The yellow-orange precipitate was dissolved in ether and partitioned between $NaHCO_3$, Na_2CO_3 and NaOH. The extracted ether solution was evaporated to dryness and the residue on crystallization from methanol gave unchanged atrovenetin yellow trimethyl ether (80 mg.).

The $NaHCO_3$ extract was acidified and the yellow precipitate was collected and washed with ether, giving a yellow crystalline solid (45 mg.), m.p. 290°. Recrystallization from methanol gave the *oxidation product* as pale-yellow needles (36 mg.), m.p. 306–307°. (Found: C, 63·8; H, 5·21; OCH_3, 24·1; *C*-CH_3, 7·7. $C_{22}H_{22}O_8$ requires C, 63·75; H, 5·35; 3 OCH_3, 22·5; 2*C*-CH_3, 7·3 %.) The substance gives a red-brown ferric colour in ethanol It dissolves only slowly in $NaHCO_3$ but is readily soluble in Na_2CO_3.

The Na_2CO_3 and NaOH extracts gave only negligible amounts of material.

(ii) *With* CrO_3. A solution of CrO_3 (0·5 g.) in water (3 ml.) and acetic acid (20 ml.) was added during 1 hr. to a solution, heated at 100°, of atrovenetin yellow trimethyl ether (0·5 g.) in acetic acid (10 ml.). The solution was held at 100° for a further hour. The resulting green solution was evaporated *in vacuo* giving a partially crystalline residue which was diluted with methanol, and the solid (112 mg.), m.p. 263–264°, was collected. Crystallization from acetic acid–water or $CHCl_3$–light petroleum (b.p. 60–80°) gave the CrO_3 *oxidation product* as yellow-brown prisms, m.p. 277–278°. (Found: C, 66·5, 66·6; H, 5·16, 5·26; OCH_3, 9·2. $C_{19}H_{18}O_6$ requires C, 66·7; H, 5·30; 1 OCH_3, 9·1 %.) The compound was recovered unchanged after heating for 2 hr. at 100° with acetic anhydride and anhydrous sodium acetate. It gives a precipitate with Brady's reagent.

A solution of the oxidation product (50 mg.) and hydroxylamine hydrochloride (50 mg.) in pyridine (about 1 ml.) was heated at 100° for 2 hr. and was then poured into water. The product was crystallized from ethyl acetate giving the *oxime of the oxidation product* (11 mg.) as brown-orange rosettes of needles, m.p. 221°. (Found: C, 63·5; H, 5·45. $C_{19}H_{19}O_6N$ requires C, 63·8; H, 5·36 %.)

(iii) *With selenium dioxide.* Mixtures of the trimethyl ether (0·5 g. in each case) with SeO_2 (0·2 g. freshly sublimed) were boiled in (*a*) acetic anhydride (5 ml.) for 6 hr. and (*b*) pentanol (10 ml.) for 24 hr. Unchanged trimethyl ether was recovered in 32·6 % yield in case (*a*) and in 86·6 % in case (*b*). The mother liquors gave positive reactions with Brady's reagent in each case, but no crystalline carbonyl compound could be isolated.

H. Zn *dust-distillation of atrovenetin.* An intimate mixture of finely ground atrovenetin (3 g.) and electrolytic zinc dust (150 g.) was distributed among 150 special glass tubes similar in construction to those described by Kögl & Postowsky (1924). The tubes were heated to a dull-red heat and the crystalline distillates which collected in the capillary end of the tubes were extracted with ether. After removal of the solvent, the residue was dissolved in light petroleum (b.p. 40–60°) and chromatographed on Al_2O_3, the separation being observed in u.v. light. The more rapidly moving and blue-fluorescing fraction I was collected, rechromatographed and sublimed twice *in vacuo* giving a colourless crystalline solid (3 mg.), m.p. 125–128°. It gave a crystalline picrate with ethanolic picric acid which, on recrystallization from ethanol, formed long glistening red needles, m.p. 218–220° and mixed m.p. 218–220° with authentic pyrene picrate, m.p. 222°. Its u.v.-absorption spectrum is indistinguishable from that of pyrene.

The more slowly eluted yellow band was rechromatographed and sublimed twice *in vacuo* giving fraction II as a pale-yellow solid (0·64 mg.), the ethanolic solution of which fluoresced blue.

Ultraviolet-absorption spectra

The u.v.-absorption spectra of atrovenetin, herqueinone and norherqueinone and a number of their derivatives were determined by means of a Hilger and Watts 'Uvispek' spectrophotometer with the collaboration of Dr J. H. Birkinshaw. The wavelengths (mμ.) and molar extinction coefficients (log ϵ, in parentheses) of the maxima ($\lambda_{max.}$) and also of the principal minimum ($\lambda_{min.}$) of the curves obtained are assembled below. All absorption spectra were measured in ethanolic solution.

(1) Atrovenetin. λ 222 mμ. (log ϵ, 4·52); inflexion at 250–260 mμ. (4·26); 385 mμ. (4·21); inflexion at 410–420 mμ. (4·10); $\lambda_{min.}$ 310 mμ. (2·74).

(2) Herqueinone. 220 mμ. (4·29); 250 mμ. (4·09); 314 mμ. (4·47); inflexion at 365–380 mμ. (3·50); 416 mμ. (3·66); $\lambda_{min.}$ 270 mμ. (3·89).

(3) Norherqueinone. 223 mμ. (4·41); inflexion at 251–254 mμ. (4·13); 315 mμ. (4·59); plateau at 360–390 mμ. (3·66); plateau at 410–430 mμ. (3·61); $\lambda_{min.}$ 275 mμ. (3·98).

(4) Xanthoherquein. 222 mμ. (4·47); 387 mμ. (4·23); $\lambda_{min.}$ 310 mμ. (3·31).

(5) Atrovenetin yellow trimethyl ether. 228 mμ. (4·47); inflexion at 270–275 mμ. (4·18); inflexion at 380–400 mμ. (4·31); $\lambda_{min.}$ 297 mμ. (2·51).

(6) Atrovenetin tetramethyl ether B, m.p. 186–187°. 220 mμ. (3·91); 281 mμ. (3·89); 364 mμ. (3·48); 426 mμ. (3·58); $\lambda_{min.}$ 302 mμ. (2·08).

(7) $LiAlH_4$ reduction product YA, m.p. 145–146°. 220 mμ. (4·31); 239 mμ. (4·36); 272 mμ. (4·40); 340 mμ. (3·96); 422 mμ. (4·14); $\lambda_{min.}$ 300 mμ. (2·90).

(8) $LiAlH_4$ reduction product OA, m.p. 203–204°. 220 mμ. (4·48); 275 mμ. (4·56); 342 mμ. (3·97); 438 mμ. (4·27); 462 mμ. (4·25); $\lambda_{min.}$ 295 mμ. (3·00).

(9) Colourless Zn dust distillate of atrovenetin, fraction I, ? pyrene. Inflexion at 230 mμ. (4·55); 240 mμ. (4·70); 262 mμ. (4·35); 272 mμ. (4·55); 287 mμ. (3·98); 306 mμ. (4·00); 318 mμ. (4·31); 334 mμ. (4·51).

(10) Authentic pyrene (Friedel & Orchin, 1951). 231 mμ. (4·65); 241 mμ. (4·9); 262 mμ. (4·4); 273 mμ. (4·7); inflexion at 293 mμ. (3·65); 306 mμ. (4·05); 318 mμ. (4·5); 334 mμ. (4·7).

(11) Yellow Zn dust distillate of atrovenetin, fraction II, not identified. 223 mμ.; 237 mμ.; 257 mμ.; 266 mμ.; 278 mμ.; 289 mμ.; 316 mμ.; 331 mμ.; 365 mμ.; 384 mμ.

Derivatives of xanthoherquein and norxanthoherquein

Xanthoherquein tetraacetate. Xanthoherquein (0·5 g.) was acetylated with acetic anhydride (5 ml.) and 60% $HClO_4$ (5 drops) at 100° for 1 hr. The crude brown acetate obtained was collected, dried (0·78 g.) and extracted with benzene (40 ml.), leaving a bright-yellow solid which was crystallized from ethyl acetate and gave *xanthoherquein tetraacetate* (0·26 g.) as yellow rods, m.p. 217°. (Found: C, 58·1; H, 4·48; OCH_3, 6·6; $CH_3.CO$, 37·6. $C_{23}H_{20}O_{11}$ requires C, 58·5; H, 4·27; 1 OCH_3, 6·6; 4$CH_3.CO$, 36·4%.) The product gives a positive ferric colour in ethanol and dissolves very slowly in aqueous 2 N-NaOH to an orange solution, probably owing to hydrolysis. Its yellow solution in cold conc. H_2SO_4 has a yellow-green fluorescence.

Norxanthoherquein pentaacetate. A mixture of norxanthoherquein (0·2 g.), acetic anhydride (2 ml.) and 60% $HClO_4$ (5 drops) was heated at 100° until all of the solid dissolved. The crystalline solid which separated on cooling was collected, washed and crystallized from much ethyl acetate giving *norxanthoherquein pentaacetate* as yellow needles (153 mg.), m.p. 235–236°. (Found: C, 57·5; H, 4·12; $CH_3.CO$, 47·9. $C_{24}H_{20}O_{12}$ requires C, 57·6; H, 4·03; 5$CH_3.CO$, 43·0%.) An ethanolic solution of the product, which gives a positive ferric colour, is yellow with a green fluorescence. The substance is sparingly soluble in the usual organic solvents.

Norxanthoherquein hexamethyl ether. A mixture of norxanthoherquein pentamethyl ether (0·3 g., m.p. 141°), dry benzene (30 ml.), methyl iodide (2 ml.) and freshly prepared Ag_2O (0·2 g.) was refluxed. After 8 hr. further Ag_2O (0·2 g.) and methyl iodide (2 ml.) were added and refluxing was continued for a total of 24 hr. The mixture was then evaporated to dryness and the residue was extracted with ether. The ether extract was concentrated and chromatographed on Al_2O_3. The column was eluted with ether giving a small initial green fraction which was discarded. The solvent was removed from the main eluate and the residue was extracted with light petroleum (b.p. 40–60°) from which, on evaporation, *norxanthoherquein hexamethyl ether* separated as large orange needles (71 mg.), m.p. 109–110°. (Found: C, 64·1; H, 5·87; OCH_3, 49·2. $C_{20}H_{22}O_7$ requires C, 64·15; H, 5·92; 6 OCH_3, 49·7%.) The product gives no ferric colour in ethanolic solution.

SUMMARY

1. The dried mycelium of laboratory cultures of the type strain of *Penicillium atrovenetum* G. Smith has yielded, by solvent extraction, about 15% of its weight of a complex mixture of crude colouring matters from which about half this weight of a hitherto undescribed pigment, now named atrovenetin, has been isolated in a state of purity.

2. Two other strains of *P. atrovenetum* also yield atrovenetin.

3. Atrovenetin, $C_{19}H_{18}O_6$, yellow-orange plates, m.p. 295° (decomp.), is a dextrorotatory substance having characteristic colour reactions.

4. A number of functional derivatives and degradation products of atrovenetin are described.

5. Atrovenetin is closely related structurally to the colouring matters, herqueinone and norherqueinone, of *Penicillium herquei* Bainier & Sartory, and is identical with deoxynorherqueinone.

6. Atrovenetin, herqueinone and norherqueinone are believed to be derivatives of 9-hydroxyperinaphthen-1-one.

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