Studies in the Biochemistry of Micro-organisms

74. THE MOLECULAR CONSTITUTION OF GEODIN AND ERDIN, TWO CHLORINE-CONTAINING METABOLIC PRODUCTS OF *ASPERGILLUS TERREUS* THOM. PART 3. POSSIBLE STRUCTURAL FORMULAE FOR GEODIN AND ERDIN

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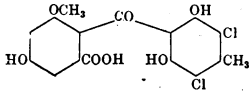
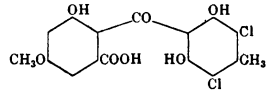
Geodin and erdin were first described by Raistrick & Smith (1936) and further characterized by Clutterbuck, Koerber & Raistrick (1937). They are two chlorine-containing metabolic products of the mould *Aspergillus terreus* Thom of empirical formulae $C_{17}H_{12}O_7Cl_2$ and $C_{16}H_{10}O_7Cl_2$ respectively. Calam, Clutterbuck, Oxford & Raistrick (1939) later synthesized the trimethyl ethers of the reduction products of these substances, showed that dihydrogeodin is the methyl ester of dihydroerdin, and proved that the latter has one of the two following structural formulae:



I

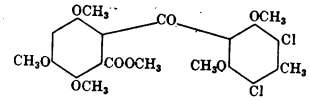


II

Of these the structure I is perhaps more likely to be correct since dihydrogeodin does not quickly give a blue coloration with 2:6-dichloroquinone-chloroimide. We are indebted to Prof. A. R. Todd for drawing our attention to this reaction which, if positive, indicates the presence of a free position *para* to a phenolic group (cf. Gibbs, 1927). Although when an immediate blue colour is given in this reaction it is usually a reliable guide it may occasionally give positive results when the *para* position is occupied (cf. Davidson, Keane & Nolan, 1943) and we have also encountered heavily substituted phenols which give a negative reaction when the *para* position is free. Examples of this are the mould metabolic products 3:5-dihydroxy-2-carboxybenzoyl methyl ketone and the corresponding carbinol (Oxford & Raistrick, 1933). 3:5-Dihydroxyphthalic acid also gives a quite atypical purplish brown colour with the reagent. Gibbs himself stated that the reaction failed in certain unspecified instances so that structure II for dihydroerdin cannot be ruled out by the negative indophenol reaction alone.

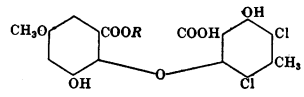
Before discussing the structures of the metabolic products themselves it will be convenient to describe some further degradation products of geodin and erdin.

(*a*) *Methylated geodin.* Clutterbuck *et al.* (1937) prepared methylated geodin, and the identical methylated erdin, by the action of methyl sulphate and alkali on the parent compounds. In the formation of methylated geodin a molecule of water is added and the resultant product contains a total of six methoxyl groups. It has now been shown that this compound has the structure III since on treatment with 80% sulphuric acid (cf. Graebe & Eichengrun, 1892) it yielded 2:6-dichloro-3:5-dimethoxy-*para*-orsellinic acid and a dimethoxyhydroxybenzoic acid which on methylation gave the known 2:3:5-trimethoxybenzoic acid.



III

(*b*) *Geodin and erdin hydrates.* Corresponding products from erdin and geodin were obtained by treatment of the parent compounds with 80% sulphuric acid. Under these conditions there are obtained the dibasic *erdin hydrate* and its monomethyl ester *geodin hydrate*, identical compounds being obtained therefrom on full methylation or by ethylation followed by hydrolysis. These compounds give a violet colour with ferric chloride and a blue colour with 2:6-dichloroquinone-chloroimide, and their structures are represented almost certainly by IV, for the following reasons.



IV. Erdin hydrate: $R=H$. Geodin hydrate: $R=CH_3$.

Fully methylated geodin hydrate has the molecular formula $C_{20}H_{20}O_8Cl_2$; twelve of the carbon atoms are accounted for by the two benzene rings,

two by carboxyl groups, five by methoxyl groups and one by a methyl side chain. The above groups also account for all twenty hydrogen atoms and seven oxygen atoms, while the two chlorine atoms will be attached to the benzene ring in the usual way. The remaining atom is a single oxygen which can reasonably be placed only as a bridge between the two rings. The structure IV is in accordance with the colour reactions mentioned above.

(c) *Miscellaneous reactions.* As was pointed out by Raistrick & Smith (1936) geodin is optically active while erdin is not. As there was a possibility that erdin might have been racemized during isolation two attempts were made to resolve it, using brucine and *d*-α-phenylethylamine. Both attempts were unsuccessful and it was concluded that erdin probably contains no asymmetric centre.

Although the carbonyl groups of the dihydro derivatives of geodin and erdin are not reactive geodin readily forms an oxime. This reaction shows that geodin, and presumably erdin, probably contain a second keto group. From this and the fact that geodin and erdin form addition compounds with diazomethane (Raistrick & Smith, 1936) it may be inferred that, although almost colourless, they may still contain a quinonoid or potential quinonoid structure in the non-chlorinated ring. The absorption spectra of geodin, erdin and their dihydro derivatives are not incompatible with the presence of such a structure. In alcoholic solution their main features are strong absorption at the shorter wavelengths followed by a peak at 275–285 mμ. After this the absorption falls off to a low value at about 360 mμ. There is no evidence of a peak in the visible region that would be expected if a true quinone were present.

The actual peaks fell thus:

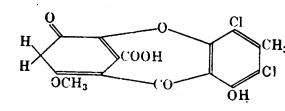
geodin	284 mμ; Σ 15,000
erdin	274 mμ; Σ 16,000
dihydrogeodin	279 mμ; Σ 20,000
dihydroerdin	285 mμ; Σ 16,000

When dihydrogeodin or dihydroerdin is boiled with hydriodic acid fission of the molecule takes place and α-resorcylic acid and orcinol can be isolated (Calam *et al.* 1939). When geodin or erdin are similarly treated no fission of the molecule occurs, one molecule of carbon dioxide is evolved and from the mother liquor two isomeric non-chlorinated substances can be isolated. These substances, nor*geodin A* and nor*geodin B* each have the composition $C_{14}H_{10}O_5 . H_2O$ and on treatment with diazomethane, di-ethers, and with dimethyl sulphate tri-ethers, of $C_{14}H_{10}O_5$ in each instance are produced. The structure of *nor*geodin B is obscure, but the yellow *nor*geodin A is almost certainly a xanthone since its trimethyl ether was soluble in conc. HCl and readily formed a ferrichloride.

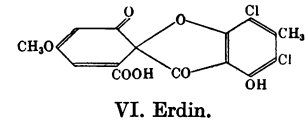
DISCUSSION

Constitution of geodin and erdin

The above evidence is sufficient to limit the reasonable structures for erdin to two only. The formation of the hydrates (IV) with sulphuric acid and the methylation product (III) obtained with dimethyl sulphate and alkali indicate the presence of an oxygen bridge between the two six-carbon rings in addition to the carbonyl group already demonstrated, but afford no certain indication of its point of attachment, relative to the —CO—, to the non-chlorinated ring. The reactions with hydroxylamine and with diazomethane suggest the presence of a quinonoid or potential quinonoid structure. On this basis we suggest for erdin the alternative structures V and VI.



V. Erdin.



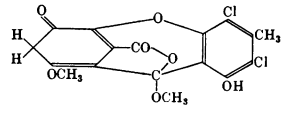
VI. Erdin.

The principal reactions of erdin can be readily explained by these structures. On hydrogenation the oxygen link presumably breaks yielding I and II respectively. On treatment of erdin with dimethyl sulphate and alkali a similar fission appears to occur but with the formation of a new hydroxyl group on the α-resorcylic acid nucleus. With either V or VI this would appear at the 2 position, since in the latter case the OH group might migrate from the point of attachment in accordance with the known reactions of quinonoid systems. On acetylation under mildly acidic conditions a similar reaction presumably occurs analogous to the Thiele acetylation of quinones (cf. Clutterbuck *et al.* 1937). With strong acid, not the —O— but the carbonyl link breaks giving the diphenyl ether IV. This reaction rules out the *para* quinonoid structure corresponding to VI since the product (geodin or erdin hydrate) gives a positive reaction at once with 2:6-dichloroquinonechloroimide. In the above reactions it is assumed that the non-aromatic structure reverts automatically to the corresponding phenolic structure when one of the links between the rings is broken. Either structure will serve to explain the formation of a xanthone, *nor*geodin A, by rearrangement after reductive decarboxylation.

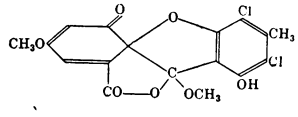
In criticism of these structures it may be said of (V) that, although it readily explains the chemical reactions of erdin, and contains no asymmetric carbon atom, there is really nothing to prevent the non-chlorinated ring from changing to the phenolic form with loss of ketonic properties. The seven-membered ring is also unorthodox but this is hardly a valid reason for rejecting it, since a not unduly strained three-dimensional model of structure (V) can be constructed. Structure (VI) should be stable, but is unsatisfactory in so far as the dihydro compound derived from it would be a phenol with a free *para* position and it would have to be assumed that the negative outcome of the indophenol reaction was due to the heavy substitution of the phenolic ring as mentioned earlier. Structure (VI) also contains an asymmetric carbon atom and hence erdin should be resolvable; as mentioned above efforts to effect such a resolution failed. To sum up, on present evidence it seems that erdin must be either (V) or (VI) but there is at the moment no certain means of distinguishing between them.

Geodin appears to be best regarded as one form of the methyl ester of erdin, and we are greatly indebted to Dr C. H. Hassall for suggesting the *pseudo*-ester structures (VII) and (VIII) based on (V) and (VI) respectively. Stable *pseudo*-esters of *o*-benzoylbenzoic acid are known (Meyer, 1904, 1907) and must inevitably contain at least one asymmetric carbon atom; hence on this formulation there is no difficulty in accounting for the optical activity of geodin, even if structure (V) is assumed for erdin. Raistrick & Smith (1936) found that the treatment of geodin and erdin with diazomethane yielded isomeric but not identical products. The assumption that geodin is a *pseudo*-ester provides a satisfactory explanation for this behaviour also.

The possible presence of a *pseudo*-ester grouping in a natural product is of considerable interest because it has been found that while erdin (the γ-keto acid) shows no antibiotic activity, geodin (the supposed *pseudo*-ester) is antagonistic to the growth of many micro-organisms. The results of tests with a number of bacteria are recorded in an appendix to this memoir.



VII. Geodin.



VIII. Geodin.

EXPERIMENTAL

Hydrolytic fissions

(i) *Of geodin tetramethyl ether.* The compound (1·006 g.), prepared as described by Clutterbuck *et al.* (1937) was heated in a stream of nitrogen with water (10 ml.) and conc. H_2SO_4 (20 ml.). At a bath temperature of 150° the colour of the solution changed, the oil bath was removed and the flask allowed to cool. The nitrogen was passed into a bubbler of standard baryta solution, and the CO_2 produced was equivalent to 7·4 ml. 0·1 N-NaOH.

After adding 25 ml. water the flask was cooled and the solid filtered off and ground with saturated $NaHCO_3$ solution. The $NaHCO_3$ insoluble part was purified by subliming in high vacuum and was proved to be 2:6-dichloro-orcinol dimethyl ether by its mixed m.p. of 130–131°. (Yield: 0·08 g.; calc. from CO_2 produced 0·075 g.) The bicarbonate-soluble part was precipitated by acid, and the solid (0·29 g.) purified by sublimation and crystallization from aqueous ethanol; colourless hair-like needles were obtained, m.p. 201°, which were found to be identical with synthetic 2:6-dichloro-3-hydroxy-5-methoxy-*p*-toluic acid (cf. Calam & Oxford, 1939).

The original H_2SO_4 mother liquor was extracted continuously with ether for 6 hr. On evaporation 0·21 g. of crystals remained behind. Purified by subliming in high vacuum they gave an intense royal blue with $FeCl_3$, a negative Millon reaction, a negative halogen test, were acid to litmus and melted at 176–180°. Found: OCH_3, 31·55; a *dimethoxyhydroxybenzoic acid* requires OCH_3, 31·31%. On treatment with dimethyl sulphate and alkali in acetone under the usual conditions, 2:3:5-trimethoxybenzoic acid was obtained, m.p. 101–102°, identical in all respects with a synthetic sample prepared from 5-hydroxyhemipinic acid as described by Faltis & Kloiber (1929). (Found: C, 56·56, 56·72; H, 5·67, 5·77; OCH_3, 43·35. $C_{10}H_{12}O_5$ requires C, 56·59; H, 5·70; OCH_3, 43·83%.)

(ii) *Of geodin and erdin. The production of geodin and erdin hydrates.* Geodin (0·5 g.) was heated on the boiling water bath for 5 min. with conc. H_2SO_4 (10 ml.) and water (5 ml.) and then kept overnight. Water (100 ml.) was added and the product filtered and dried, giving a pale yellow solid which was extracted with hot chloroform to remove unchanged geodin. The residue was purified by crystallizing from aqueous ethanol which gave very pale yellow prisms, m.p. 209°. With $FeCl_3$ an intense violet colour was produced and with 2:6-dichloroquinone-chloroimide an almost immediate pure blue colour as with phenol itself. (Found: C, 49·03, 48·82; H, 3·55, 3·58; Cl, 16·9, 16·8; OCH_3, 15·42, 15·3. $C_{17}H_{14}O_8Cl_2$ requires C, 48·96; H, 3·39; Cl, 17·00; OCH_3, 14·87%.) Erdin, treated in the same way, gave a khaki-coloured solid forming pale brown parallelograms from aqueous ethanol, m.p. 222–223°. With $FeCl_3$ a violet colour is produced. (Found: C, 47·86, 47·87; H, 3·25, 3·20; OCH_3, 8·43. $C_{16}H_{12}O_8Cl_2$ requires C, 47·66; H, 3·00; OCH_3, 7·69%.)

Methylation

Erdin hydrate (0·53 g.) was shaken with 5 ml. dimethyl sulphate and 10 ml. acetone, and eight successive lots of 5 ml. 2 N-NaOH were added at intervals of 5 min. A glassy solid remained after the liquid had been decanted, and this formed colourless needles from aqueous ethanol,

m.p. 120–121°, giving no colour with $FeCl_3$. (Found: C, 51·90, 52·17; H, 3·94, 4·06; Cl, 15·45; OCH_3, 35·1; CH_3-C (Kuhn-Roth), 2·99. $C_{20}H_{20}O_8Cl_2$ requires C, 52·30; H, 4·39; Cl, 15·44; $(OCH_3)_5$, 33·8; CH_3-C, 3·27%.)

Hydrolysis. The above compound (0·1 g.) was refluxed 1 hr. with ethanol (10 ml.) and N-NaOH (5 ml.). After removing the ethanol *in vacuo* and acidifying, the product (0·07 g.) was crystallized from ethyl acetate/light petroleum when it formed colourless needles, m.p. 239–240°. (Found: OCH_3, 21·09; equivalent, 220. $C_{18}H_{16}O_8Cl_2$ requires $(OCH_3)_3$, 21·57%; equiv. (as a dibasic acid), 215·5.)

Ethylation and hydrolysis of geodin hydrate

Geodin hydrate (0·5 g.) was refluxed during 4½ hr. with sodium (0·12 g.), ethyl iodide (5 ml.) and ethanol (15 ml.), when the solution became neutral. The solvents were removed as far as possible, and the residue hydrolyzed with 2N-NaOH (15 ml.) and ethanol (15 ml.). After distilling off the ethanol, filtering and acidifying, a solid separated (0·38 g.). It formed rosettes of needles from ethyl acetate/light petroleum, m.p. 206–207°. (Found: C, 51·47; H, 4·53; Cl, 15·2; OCH_3, 20·8. $C_{20}H_{20}O_8Cl_2$ requires C, 52·30; H, 4·39; Cl, 15·44; $(OC_2H_5)_2 + OCH_3$, calc. as $(OCH_3)_3$, 20·25%.)

Erdin hydrate was treated in the same way and gave identical rosettes of needles, whose m.p. was not depressed on mixing with the above product from geodin hydrate.

Reaction of geodin and erdin with HI

Geodin (1·0382 g.) was heated in a Zeisel flask with HI (*d*, 1·7) (15 ml.) in a stream of CO_2-free nitrogen, and the gases were passed through ethanolic $AgNO_3$ and standard baryta water. The bath temperature was 140–150°. CO_2 was evolved equivalent to 51·65 ml. 0·1N-NaOH (calc. for 1 CO_2 per mol., 52·0 ml.). From the $AgNO_3$ solution was obtained 1·167 g. AgI (calc. 1·22 g. AgI for $2OCH_3$). The contents of the Zeisel flask were poured into water and the solid filtered off (0·68 g., calc. 0·72 g.). This mixture consists of two isomerides, *nor*geodin A and *nor*geodin B, and they can be separated either by precipitating from warm acetone with 2 vols. chloroform, when the less soluble *nor*geodin B crystallizes out; or better, by extracting the soluble A with warm glacial acetic acid. About 10 parts of B are present to 1 of A.

Nor*geodin A*, crystallized repeatedly from aqueous ethanol, formed bright yellow needles (with 1 mol. H_2O) with a max. m.p. 305° dec., darkening commencing at 275°. It gives an intense green colour with $FeCl_3$. It has no acidic properties. (Found: C, 60·89; H, 4·41. $C_{14}H_{12}O_6$ requires C, 60·87; H, 4·38%.)

Nor*geodin B* formed pale yellow needles from acetone/chloroform, max. m.p. 325–330° dec., darkening beginning at 300°. It gives no colour with $FeCl_3$, and is free from Cl. (Found: C, 60·76, 60·99; H, 4·27, 4·44. $C_{14}H_{12}O_6$ requires C, 60·87; H, 4·38%.) It is slowly soluble in $NaHCO_3$ solution. Erdin, treated in the same way, gave the same two products as did geodin, their identity being shown by comparing their trimethyl derivatives.

Methylation products

(*a*) *With dimethyl sulphate.* *Nor*geodin A (0·15 g., m.p. 305° dec.) was treated with dimethyl sulphate in acetone and NaOH in the usual way. The trimethyl ether separated out and was filtered off (0·15 g.). From ethanol containing a little water were obtained very pale yellow needles, m.p. 216–217°, which gave no colour with $FeCl_3$. (Found: C, 67·57, 67·52; H, 5·13, 5·31; OCH_3, 30·8, 30·7. $C_{17}H_{16}O_5$ requires C, 68·00; H, 5·37; $(OCH_3)_3$, 30·97%.)

That it is likely that *nor*geodin A is a xanthone is shown by the fact that the above trimethyl ether slowly dissolves in conc. HCl, in the same way as does xanthone itself. On adding $FeCl_3$ to such a solution a red *ferrichloride* separates. On crystallizing from hot glacial acetic acid its m.p. can be raised to 181° (decomp.), but after a few recrystallizations it begins to decompose. (Found: Fe, 10·8, 10·8; calc., 11·2%.)

*Nor*geodin B (0·35 g., giving no colour with $FeCl_3$) was methylated as above. The colourless product which separated formed needles from ethanol, and melted at 198·5–199·5°. The ethanolic solution, like that of *nor*geodin A trimethyl ether, shows a marked bluish fluorescence. (Found: C, 67·79, 67·67; H, 5·17, 5·30; OCH_3, 30·8, 31·3. $C_{17}H_{16}O_5$ requires C, 68·00; H, 5·37; $(OCH_3)_3$, 30·97%.) On mixing with *nor*geodin A trimethyl ether the m.p. was depressed to 187–195°. It is quite insoluble in HCl and forms no ferrichloride.

(*b*) *With diazomethane.* *Nor*geodin A (0·4 g., m.p. 300°) was treated with an excess of ethereal diazomethane. After keeping overnight the insoluble dimethyl ether was filtered off (0·37 g.) and crystallized from ethanol. Colourless, hair-like needles were formed, m.p. 273–275° which gave an intense green colour with $FeCl_3$. (Found: C, 67·61, 67·47; H, 5·22, 5·22; OCH_3, 22·2, 21·9. $C_{16}H_{14}O_5$ requires C, 67·16; H, 4·92; $(OCH_3)_2$, 21·65%.)

*Nor*geodin B, treated similarly, gave a mixture of di- and trimethyl ethers. (Found: OCH_3, 23·3%.)

Attempted resolution of erdin

d-α-Phenyl-ethylamine was obtained by combining the *dl*-base with *l*-malic acid and crystallizing three times from water (cf. Loeven, 1905). The homogeneous product was decomposed with alkali, extracted with ether which was then evaporated to leave the base. To 0·12 g. of the base was added 2 ml. ether and then 0·48 g. erdin (1·5 mol.) in 30 ml. ether. The yellow crystalline precipitate was filtered off (0·27 g.) and crystallized twice from hot water to give yellow needles (33 mg.), m.p. 159–164° decomp. By grinding with 2N-HCl and repeatedly washing with water, the erdin was freed from the base. From dioxane/light petroleum it formed clusters of nitrogen-free pale buff prisms, m.p. 217–220° (decomp.). A 4·82% solution in dioxane showed no rotation in a 0·5 dm. tube. A similar attempt, using brucine, also failed.

Geodin oxime

Geodin (1 g.), hydroxylamine hydrochloride (0·4 g.) and sodium acetate (anhydrous, 1 g.) were refluxed in ethanol (60 ml.), during 45 min. After cooling, a red-brown solid separated which was filtered off. From ether/light petroleum it formed red crystals, m.p. 259–261°; it contained no nitrogen. On acidifying the filtrate with dilute HCl the oxime separated as a pale yellow solid (0·21 g.). From chloroform/light petroleum it formed short yellow needles, m.p. 285–286° (decomp.), and was appreciably soluble in water. An 0·57% solution in chloroform showed no optical activity in an 0·5 dm. tube. (Found: C, 49·0, 49·1; H, 3·3, 3·2; N, 3·9, 3·9; Cl, 17·4. $C_{17}H_{13}O_7Cl_2N$ requires C, 49·3;

H, 3·2; N, 3·4; Cl, 17·15%.) When dihydrogeodin was treated with hydroxylamine hydrochloride under identical conditions it was recovered unchanged, and the crude product contained no nitrogen.

SUMMARY

1. Further degradative studies of geodin and erdin, two chlorine-containing metabolic products of *Aspergillus terreus* Thom, are described leading to definitive structures for the methylation product obtained with dimethyl sulphate and alkali; and for geodin and erdin hydrates, obtained by the action of 80% sulphuric acid.

2. On the basis of this and other information possible structural formulae for the two metabolic products are presented, and their limitations discussed, the chief difficulties being the point of attachment of a second ether link between the rings in a highly substituted *o*-benzoylbenzoic acid, and the apparent lack of an asymmetric centre in erdin.

3. The optical activity of geodin probably lies in a *pseudo*-ester group derived from this structure, while the non-chlorinated ring in each substance has semi-quinonoid rather than phenolic properties.

The authors offer their best thanks to Prof. A. R. Todd, F.R.S., and Dr C. H. Hassall for invaluable criticism and advice, and to Prof. F. L. Bates, Physics Department, University College, Nottingham, for the use of a spectrophotometer.

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Appendix. Antibacterial Activities of Geodin and Erdin

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Methods

A sterile clear 0·4% solution of geodin was prepared by dissolving geodin (40 mg.) in 0·1 N-NaOH (2·01 ml.) and diluting with sterile 2% glucose broth medium (8 ml.). It was used immediately for making the higher dilutions in glucose broth (for tests with all strains of bacteria save one) and in plain heart broth (for test with *Vibrio cholerae* only). For every test, ten serial dilutions were prepared (see Table 1), and the tubes were immediately inoculated with one loopful of a 24 hr. culture of the desired micro-organism. Growth readings were usually taken after 24 hr. incubation at 37°, but the period was 48 hr. for strains of *Corynebacterium diphtheriae* and 65 hr. for mycobacteria. Further tests with mycobacteria were also carried out using nutrient agar instead of liquid glucose broth. In the case of erdin dilutions were made from a 1% solution prepared similarly.

Results

Geodin had no appreciable effect, at a concentration of 0·1% upon the following nine strains of Gram-negative bacteria: *Escherichia coli* (two strains); *Bacterium dysenteriae* Flexner; *Bact. dysenteriae* Sonne; *Salmonella paratyphi* B; *Salm. typhimurium*; *Salm. enteritidis*; *Proteus vulgaris*, and upon one Gram-positive organism (*Mycobacterium phlei*, N.C.T.C. No. 525). It had an appreciable inhibitory effect upon the growth of nineteen Gram-positive strains (including two non-pathogenic strains of mycobacteria) and upon two Gram-negative organisms (*V. cholerae* and *Bact. dysenteriae* Shiga), at limiting dilutions varying from 1 : 1000 to 1 : 128,000 (see Table 1). Erdin had not the slightest inhibitory effect upon any of the above micro-organisms when present at a concentration of 0·1%.

SUMMARY

Geodin may be regarded as a moderately potent antibiotic substance active in the main against Gram-positive bacteria only, while erdin has not been observed to inhibit the growth of bacteria.

Table 1. *Bacteriostatic power of geodin against nineteen Gram-positive and two Gram-negative strains in glucose broth*

Micro-organism	Catalogue No. (National Collection of Type Cultures) *or* London School of Hygiene and Tropical Medicine	Limiting dilution for complete inhibition of growth
Bacterium dysenteriae Shiga	N.C.T.C. 4837	1,000
Vibrio cholerae	N.C.T.C. 1633	2,000 (in plain broth)
Mycobacterium smegmatis	N.C.T.C. 523	2,000
Staphylococcus aureus	N.C.T.C. 4736	4,000
Staph. aureus	N.C.T.C. 3093	8,000
Staph. albus	N.C.T.C. 3256	8,000
Streptococcus viridans	N.C.T.C. 3166	8,000
Myco. smegmatis	N.C.T.C. 523	8,000 (in nutrient agar)
Staph. aureus	N.C.T.C. 4163	16,000
Mycobacterium sp. (saprophytic)	N.C.T.C. 510	16,000
Staph. aureus	N.C.T.C. 3750	32,000
Bacillus subtilis	L.S.H.	32,000
B. anthracis	L.S.H.	32,000
Corynebacterium diphtheriae gravis	L.S.H.	32,000
C. diphtheriae intermedius	L.S.H.	32,000
Staph. aureus	L.S.H.	64,000
Staph. aureus	N.C.T.C. 3761	64,000
Staph. aureus	N.C.T.C. 3095	64,000
C. diphtheriae mitis	L.S.H.	64,000
Staph. citreus	N.C.T.C. 2301	128,000
Strep. pyogenes Griffith type I	N.C.T.C. 2432	128,000
B. anthracis	N.C.T.C. 5444	128,000

Studies on Antibiotics*

2. BACTERIOLOGICAL ACTIVITY AND POSSIBLE MODE OF ACTION OF CERTAIN NON-NITROGENOUS NATURAL AND SYNTHETIC ANTIBIOTICS

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In our search for new antimicrobial agents of natural origin, Dr R. Klein, in our laboratories, found some time ago that the crude culture fluid of *Aspergillus terreus*, strain N.C.T.C. 3911 (Ac 100), possessed a far higher antibiotic activity than could be accounted for by its known content of citrinin (Raistrick & Smith, 1935; 1936). We succeeded in isolating in addition to the latter substance a crystalline, faintly yellow compound which had marked antibiotic activity; this compound was examined chemically by Mr C. H. Hassall and Prof. A. R. Todd of Cambridge University who identified it as geodin, $C_{17}H_{12}O_7Cl_2$, previously isolated from another organism by Raistrick & Smith (1936).

While still engaged on the elucidation of the structure of clavatin or patulin (Bergel, Morrison, Moss & Rinderknecht, 1944; cf. also Raistrick, 1943) we also synthesized a number of compounds possessing some of the structural features of penicillic acid (Birkinshaw, Oxford & Raistrick, 1936) and of the clavatin molecule, which we knew at the time to be an unsaturated keto lactone.

In the following we present the preliminary results of the bacteriological tests and experimental evidence for a possible mode of action of these substances. Numbers following compounds refer to Table 2, where formulae are given.

* Part I, see *J. chem. Soc.* (1944), p. 415.

METHODS AND EXPERIMENTAL

Natural compounds (mycological investigations by R. Klein)

Penicillic acid and clavatin (nos. 1 and 2) were prepared as described by Birkinshaw *et al.* (1936) and Bergel, Morrison, Moss, Klein, Rindernecht & Ward (1943) respectively. For the preparation of geodin (no. 3) from *Aspergillus*