CXLIII. STUDIES IN THE BIOCHEMISTRY OF MICRO-ORGANISMS

LV. THE MOLECULAR CONSTITUTION OF GEODIN AND ERDIN, TWO CHLORINE-CONTAINING METABOLIC PRODUCTS OF *ASPERGILLUS TERREUS* THOM.

PART I. THE CONSTITUTIONAL RELATIONSHIP OF GEODIN AND ERDIN

By PERCIVAL WALTER CLUTTERBUCK, WALTER KOERBER
AND HAROLD RAISTRICK

*From the Division of Biochemistry, London School of Hygiene and Tropical
Medicine, University of London*

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In a recent communication [Raistrick & Smith, 1936] it was shown that a strain of *Aspergillus terreus* Thom, when grown on Czapek-Dox solution containing KCl as the sole source of chlorine, produced two new mould metabolic products, *geodin*, $C_{17}H_{12}O_7Cl_2$, i.e. $C_{15}H_6O_5Cl_2(OCH_3)_2$, and *erdin*, $C_{16}H_{10}O_7Cl_2$, i.e. $C_{15}H_7O_6Cl_2(OCH_3)_3$, and it was proved that these substances were true metabolic products and not artefacts.

We have now shown that these two compounds are closely related in structure, *geodin* being the dextrorotatory isomeride of a monomethyl ether of the optically inactive *erdin*.

As reported in the earlier paper, *geodin* and *erdin*, on treatment with diazomethane, give compounds having the same empirical formula,

the melting-points being 151° (decomp.) and 154° (decomp.) respectively. The mixed M.P. of these compounds gave, however, a depression to 142 – 144° . This is undoubtedly due to the experimentally established fact that the *geodin* compound is optically active, whilst the *erdin* compound is not. In the compound from *geodin* one methoxyl group, in that from *erdin* two methoxyl groups, have been added, whilst in both compounds union with a molecule of diazomethane has occurred, presumably at a double bond.

It was also shown in the earlier paper that both *geodin* and *erdin* absorb 1 mol. of hydrogen giving dihydro-derivatives, and that in the case of *geodin*, loss of optical activity occurred during the process. The dihydro-derivatives were, therefore, methylated in the hope that both dihydro*geodin* and dihydro*erdin* would give the same compound. This proved to be the case, dihydro*geodin* with diazomethane adding three, and dihydro*erdin* adding four methoxyl groups, the products both having the same empirical formula, $C_{15}H_5O_2Cl_2(OCH_3)_5$, the same M.P. and showing no depression of M.P. on mixing. It will be noted that diazomethane did not form an addition product with these dihydro-derivatives. Both methylated dihydro*geodin* and methylated dihydro*erdin*, on hydrolysis with dilute NaOH in alcoholic solution, lost one methoxyl group and gave rise to the same monobasic acid, $C_{15}H_6O_3Cl_2(OCH_3)_4$.

The view that geodin and erdin have the same basal structure was further confirmed by the fact that both compounds, on methylating in alkaline solution with methyl sulphate, gave the same methyl derivative. In this case, a molecule of water is added to each molecule, geodin is rendered optically inactive, geodin adds four and erdin five methoxyl groups, each product having the formula $C_{15}H_4O_2Cl_2(OCH_3)_6$ having the same m.p. and showing no depression of m.p. on mixing. Both methylated geodin and methylated erdin, on hydrolysis with dilute NaOH in alcoholic solution, lost one methoxyl group and gave rise to the same monobasic acid $C_{15}H_5O_3Cl_2(OCH_3)_5$.

On acetylation, geodin forms a tetraacetate, the empirical formula for which $(C_{25}H_{22}O_{12}Cl_2 \equiv C_{17}H_{12}O_7Cl_2 + H_2O + 4COCH_3)$ indicates the addition of the elements of water during acetylation, thus recalling the behaviour of geodin on methylation with methyl sulphate, when a tetramethyl derivative is formed, and the elements of water are added. On the other hand, dihydrogeodin on acetylation gives a triacetate, $C_{23}H_{20}O_{10}Cl_2$, without the addition of the elements of water, just as dihydrogeodin, on methylation with diazomethane, forms a trimethyl derivative also without the addition of the elements of water.

Further work is in progress on the molecular structure of geodin and erdin, and will be reported at a later date.

EXPERIMENTAL

Methylation of dihydrogeodin and of dihydroerdin with diazomethane. It has been shown [Raistrick & Smith, 1936] that geodin, which is dextrorotatory, on treatment with $Pd-C-H_2$ in alcoholic solution, absorbs H_2 and gives dihydrogeodin, which is optically inactive. This compound was prepared and methylated with diazomethane in ether and after removal of solvent, the methylated product solidified. On recrystallization from light petroleum, B.P. 50–60°, it separated in colourless needles, m.p. 108°. (Found (Weiler): C, 54.11, 54.13; H, 4.49, 4.42; Cl, 16.03, 15.94; N, nil; OCH_3 (macro), 34.1%. $C_{15}H_5O_2Cl_2(OCH_3)_5$ requires C, 54.16; H, 4.55; Cl, 16.03; $5OCH_3$, 35.0%.) The compound therefore contains five methoxyl groups and diazomethane had thus inserted three additional methoxyl groups. The same compound (m.p. 108°) was obtained from dihydroerdin on methylation with ethereal diazomethane, four additional methoxyl groups being inserted. Methylated dihydrogeodin did not depress the m.p. of methylated dihydroerdin.

Hydrolysis of methylated dihydrogeodin and of methylated dihydroerdin. Methylated dihydrogeodin (560.3 mg.) was dissolved in alcohol (50 ml.) and *N* NaOH (25 ml.) added. After boiling under a reflux condenser for 1 hr. and titrating back, 1.30 ml. *N* NaOH had been used (removal of one OMe group requires 1.26 ml.). After removing alcohol and acidifying, 510 mg. of a dry acid, m.p. 168°, were obtained, which on recrystallization from alcohol-water separated in colourless rectangular prisms, m.p. 168°, readily soluble in $NaHCO_3$. (Found (Weiler): C, 52.94, 53.02; H, 4.24, 4.41; Cl, 15.8, 16.0; OMe, 28.1, 28.5%. $C_{15}H_6O_3Cl_2(OMe)_4$ requires C, 53.13; H, 4.23; Cl, 16.55; OMe, 28.9%.) 49.8 mg. of this acid, on titration to phenolphthalein, required 1.18 ml. *N*/10 NaOH, corresponding with an equivalent of 422 ($C_{19}H_{18}O_7Cl_2$, titrating as a monobasic acid, requires 429). Methylated dihydroerdin was hydrolysed in the same way as methylated dihydrogeodin and gave the same acid having the same m.p., mixed m.p. and equivalent and the same general properties.

One methoxyl group in methylated dihydrogeodin and in methylated dihydroerdin is thus hydrolysable by heating with NaOH in alcoholic solution and is therefore probably present as a methyl ester group.

Methylation of geodin and erdin in alkaline solution with methyl sulphate. Geodin (0.5 g.) was dissolved in acetone (10 ml.) and methyl sulphate (5 ml.) was added. 2*N* NaOH (8 lots of 5 ml. each) was added at intervals with shaking. Geodin readily dissolved in NaOH but, as methylation neared completion, a very pale yellow crystalline methylation product separated from the alkaline solution and was filtered, washed and dried; m.p. 147°. After boiling in methyl alcoholic solution with a little charcoal, filtering and adding a little water, it crystallized in colourless needles, m.p. 150°. It did not lose weight on drying at 60° in a high vacuum. (Found: C, 53.88; H, 4.69; Cl, 15.0; 14.7; OCH₃ (macro) 38.0%. C₁₅H₄O₂Cl₂(OMe)₆ requires C, 53.25; H, 4.69; Cl, 15.01; 6OCH₃, 39.3%.)

The substance was optically inactive. Since

it appears that, on methylation in alkaline solution with methyl sulphate, geodin adds 1 mol. H₂O, and four OH groups are methylated.

The behaviour of erdin on methylation in alkaline solution with methyl sulphate was exactly similar to that of geodin, the two methylation products having the same general properties, m.p. and mixed m.p.

Hydrolysis of methylated geodin and of methylated erdin. Methylated geodin (0.4168 g.) prepared as described above was hydrolysed. It was dissolved in alcohol (50 ml.), *N* NaOH (25 ml.) was added and the solution heated on the water-bath for 2 hr. On back-titration, 0.95 ml. *N* NaOH had been used: (the loss of one OMe group requires 0.88 ml.). After removal of alcohol the titrated solution was acidified. A crystalline precipitate separated, which was filtered and dried, and then crystallized from alcohol containing a little water in long colourless needles, m.p. 163°. (Found (Weiler): C, 52.50, 52.60; H, 4.53, 4.67; Cl, 14.8, 15.2; OCH₃ (macro), 31.8%. C₁₅H₅O₃Cl₂(OMe)₅ requires C, 52.28; H, 4.39; Cl, 15.46; 5OCH₃, 33.8%.) 0.1076 g. required 2.35 ml. *N*/10 NaOH for neutralization to phenolphthalein, corresponding with an equivalent of 458. (C₂₀H₂₀O₈Cl₂, titrating as a monobasic acid, requires 459.) It appears, therefore, that of the six OMe groups of methylated geodin, one is readily hydrolysable by *N* NaOH.

The acid obtained on hydrolysis of methylated erdin had the same general properties, the same m.p., mixed m.p. and equivalent as that obtained, as described above, from methylated geodin.

Acetylation of geodin (with Mr G. Smith). Geodin (1 g.) was heated at 140–150° for 20 min. with sodium acetate (2 g.), acetic anhydride (4 ml.) and glacial acetic acid (6 ml.). The mixture was cooled and water was added gradually until a turbidity appeared, the whole being then poured into excess water. The product obtained by this method was less sticky than that obtained by pouring directly into excess water. It was ground with water until no longer sticky, then filtered and washed; wt. 0.79 g. On recrystallization from glacial acetic acid containing 1% of acetic anhydride it separated in minute colourless plates, m.p. 209–210°. (Found (Schoeller): C, 51.56, 51.30; H, 3.80, 4.07; Cl, 11.92%. C₂₅H₂₂O₁₂Cl₂ (≡ C₁₇H₁₂O₇Cl₂ + H₂O + 4COCH₃) requires C, 51.26; H, 3.80; Cl, 12.13%.) One molecule of water has therefore been added and a tetra-acetate formed.

Acetylation of dihydrogeodin. Dihydrogeodin (0.5 g.) was heated for 20 min. at 140–150° with sodium acetate (1 g.), acetic anhydride (2 ml.) and glacial acetic acid (3 ml.). The material was isolated as above and gave a crude product which, on crystallization from acetic acid containing 1% acetic anhydride, separated in colourless, rectangular prisms, m.p. 154°. (Found (Weiler): C, 52.49; H, 3.92; Cl, 13.57%. C₂₃H₂₀O₁₀Cl₂ (≡ C₁₇H₁₄O₇Cl₂ + 3COCH₃) requires C, 52.35; H, 3.82; Cl, 13.47%.) The compound is therefore the triacetate of dihydrogeodin.

SUMMARY

Geodin, $C_{17}H_{12}O_7Cl_2$, and erdin, $C_{16}H_{10}O_7Cl_2$, metabolic products of *Aspergillus terreus* Thom, have been shown to be closely related in structure, geodin being the dextrorotatory isomeride of a monomethyl ether of the optically inactive erdin. This follows since geodin and erdin give the same methyl ether on methylation with methyl sulphate in alkaline solution, and dihydrogeodin and dihydroerdin give the same methyl ether on methylation with ethereal diazomethane.

REFERENCE

Raistrick & Smith (1936). *Biochem. J.* **30**, 1315.