COMPLETE SYNTHESIS OF ANGOLIDE

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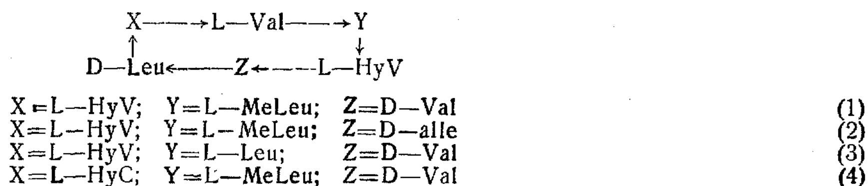
In 1963, New Zealand scientists showed that the spores of the fungus Pithomyces chartarum — the pathogen of facial eczema of ruminants — were covered with a crystalline protective coating consisting of cyclic depsipeptides [1]. Until recently, the only substances isolated from this spore coating were the following cyclohexadepsipeptides, which are extremely similar in respect to structure — sporidesmolides I (1), II (2), III (3), and IV (4), the molecules of which are constructed of two hydroxyacid residues and four aminoacid residues [2]. We have confirmed the structure of three compounds of this group of metabolites, namely sporidesmolides I (1), II (2), and IV (4), by their complete synthesis [3]:



Recently, Russell et al. [4] have isolated a new cyclic depsipeptide called angolide from the chloroform extract of the dried spores of one of the strains of Pithomyces related to Pithomyces chartarum. Its structure was established in the following way. Vigorous acid hydrolysis of angolide led to a mixture of equimolecular amounts of L-isoleucine and D-alloisoleucine, and also to L-α-hydroxyisovaleric acid [4]. On the other hand, the hydrazinolysis of this metabolite led through the rupture of the ester bonds to a mixture of hydrazides of hydroxyacylaminoacids, the molecule of one of which was constructed from the residues of hydroxyisovaleric acid and isoleucine, while the molecule of the other contained an alloisoleucine residue in addition to a hydroxyisovaleric acid residue. This shows that angolide, in contrast to the sporidesmolides, possesses a structure with a regular sequence of hydroxyacid and aminoacid residues.

In order to solve the question of the structure of angolide, it remained to determine the size of its molecular ring, i.e., to determine its molecular weight. Since, in the case of the cyclic depsipeptides, the usual methods of determining molecular weight frequently lead to very low results [5, 6], Russell requested us to determine the molecular weight of angolide thermoelectrically. The results obtained by M. Yu. Feigina (mol. wt. 413, 422 in trifluoroacetic acid) showed that angolide is a cyclotetradepsipeptide and, consequently, its structure corresponds to formula (5).

These results have been confirmed by mass spectrograph in our Institute by N. S. Vul'fson et al. [7] (found mol. wt. 426), which, on the basis of the fragmentation characteristics of the cyclodepsipeptides that we have established previously, confirmed Russell's conclusion on the regular sequence of hydroxy- and aminoacids in the angolide molecule. In actuality, the fragmentation of angolide takes place mainly by the so-called CO_2 route which is characteristic for cyclotetradepsipeptides of regular structure, and begins with the splitting out of CO_2 with the subsequent elimination of hydroxy- and aminoacid fragments*:

In order to confirm this structure for angolide, which is unusual for the metabolites of Pithomyces, we have carried out its synthesis by a scheme analogous to that which we used previously for the synthesis of cyclotetradepsipeptides structurally related to the antibiotics of of the enniatin group [5] (see Scheme). The condensation of carbobenzoxy-D-alloisoleucine (6) and of carbobenzoxy-L-isoleucine (8) with t-butyl L-α-hydroxyisovalerate (7) by the mixed anhydride method (benzene-sulfonyl chloride in pyridine) gave the diesters (9) and (10), respectively. The first of

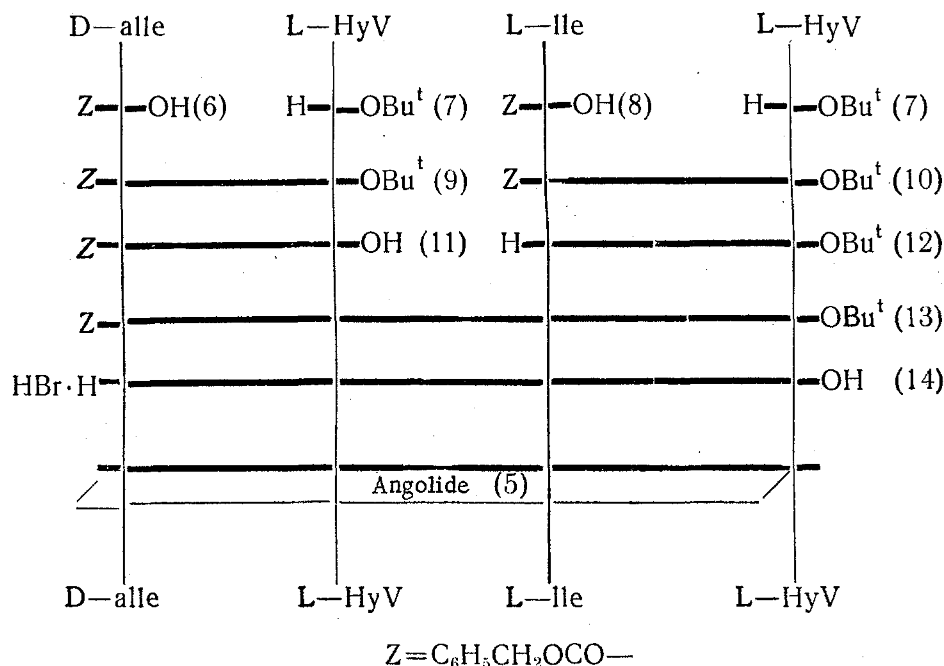
* Simultaneously, and independent of us, a mass-spectrometric investigation of angolide was carried out by Macdonald and Shannon [8]. The scheme of the fragmentation of angolide which they give coincides with our results in every detail, and completely agrees with the fragmentation characteristics of the cyclodepsipeptides that we have discovered.

these compounds was converted into the acid (11) by the action of trifluoroacetic acid, and the second, after hydrogenolysis over platinum black, gave the corresponding aminoester (12). The fragments (11) and (12) obtained in this way were linked by an amide bond by the acid chloride method (see Scheme).

The acid chloride formed by the action of PCl_5 in ether on the acid (11) was condensed with the aminoester (12) in tetrahydrofuran solution in the presence of triethylamine at -20°, giving the protected tetradepsipeptide (13). The two protective groups were removed simultaneously by the action of hydrogen bromide in glacial acetic acid, and the resulting hydrobromide of the linear tetradepsipeptide (14) was converted by means of $SOCl_2$ into the corresponding chloride. The latter, after cyclization in benzene solution in the presence of triethylamine, led to the cyclotetradepsipeptide (5) with a yield of 28% (after recrystallization from alcohol); mp 260°, $[\alpha]_D^{22}$ -83° ($CHCl_3$). This compound proved to be completely identical with natural angolide in its properties (melting point, mixed melting point, specific rotation, chromatographic behavior, IR spectrum).

The synthesis of angolide that we have effected is thus a definitive proof of its structure and forms the first synthesis of a natural cyclotetradepsipeptide constructed of α-hydroxy- and α-aminoacid residues.

Scheme



$Z = C_6H_5CH_2OCO—$

Experimental

t-Butyl carbobenzoxy-D-alloisoleucyl-L-α-hydroxyisovalerate (9). With stirring and cooling to 0°, 2.55 ml (20 mM) of benzenesulfonyl chloride was added to a solution of 5.8 g (22 mM) of carbobenzoxy-D-alloisoleucine (6) in 30 ml of dry pyridine. After 15 min, 3.31 g (19 mM) of t-butyl L-α-hydroxyisovalerate (7) was added, and the mixture was stirred for 2 hr at 0°, then for 5 hr at 20°, and then diluted with 200 ml of ether. The ethereal solution was washed with water (3 × 100 ml), 1N H_2SO_4 (2 × 100 ml), water, and saturated $NaHCO_3$ solution, and was dried with $MgSO_4$ and evaporated to dryness in vacuum. The residue was chromatographed on neutral alumina in the benzene-ethyl acetate system (gradient elution). This gave 7.0 g (87%) of the t-butyl ester (9) in the form of a colorless oil, $[\alpha]_D^{25}$ -1.4° (c 1.0; benzene).

Found %: C 65.25; H 8.30; N 3.19. $C_{23}H_{35}O_6N$. Calculated %: C 65.53; H 8.37; N 3.32.

t-Butyl carbobenzoxy-L-isoleucyl-L-α-hydroxyisovalerate (10). Under the conditions of the previous experiment, 13.3 g (50 mMole) of carbobenzoxy-L-isoleucine (7), 5.8 ml (45 mMole) of benzenesulfonyl chloride and 7.4 g (43 mMole) of t-butyl L-α-hydroxyisovalerate (8) gave 16 g (90%) of the diester (10), $[\alpha]_D^{25}$ −14°(c 1.3; benzene).

Found %: C 65.70; H 57; N 3 10. $C_{23}H_{35}O_6N$. Calculated %: C 65 53; H 8.37; N 3.32.

Carbobenzoxy-D-alloisoleucyl-L-α-hydroxyisovaleric acid (11). A solution of 4.22 g (10 mM) of the t-butyl ester (9) in 10 ml of trifluoroacetic acid was allowed to stand for 30 min, after which the solution was evaporated in vacuum, the residue treated with 10 ml of dry toluene, and again evaporated in vacuum. The residual oil was dissolved in 50 ml of ether, and the ethereal solution washed with water and extracted with 2% $NaHCO_3$ solution (3 × 20 ml). The bicarbonate extract was washed with ether (3 × 50 ml) and acidified with 2 N HCl (to Congo Red), and the

oil which separated was extracted with ether. The ethereal solution was dried with $MgSO_4$ and evaporated to dryness in vacuum. This gave 3.5 g (95%) of the acid (11) in the form of a colorless oil, $[\alpha]_D^{25}$ +9.8° (c 1.7; benzene).

Found %: C 62.40; H 7.56; N 3.98. $C_{19}H_{27}O_6N$. Calculated %: C 62.45; H 7.45; N 3.83.

t-Butyl L-isoleucyl-D-α-hydroxyisovalerate (12). A solution of 21.8 g (59 mM) of the carbobenzoxy diester (10) in 250 ml of methanol was hydrogenated (20°, 760 mm) in the presence of 1 g of palladium black until the theoretical amount of hydrogen had been absorbed (4 hr). The catalyst was filtered off, the filtrate was diluted with 600 ml of benzene, washed with a saturated solution of $NaHCO_3$, dried with $MgSO_4$, and evaporated. Fractionation of the residue in vacuum gave 10.8 g (75%) of the aminoester (12) with bp 112-114° (1 mm), $[\alpha]_D^{25}$ +2.3° (c 1.8; benzene).

Found %: C 62.55; H 10.25; N 4.67. $C_{15}H_{29}O_4N$. Calculated %: C 62.28; H 10.17; N 4.87.

t-Butyl carbobenzoxy-D-alloisoleucyl-L-α-hydroxyisovaleryl-L-isoleucyl-L-α-hydroxyisovalarte. (13). To a solution of 3.65 g (10 mM) of the acid (11) in 30 ml of dry ether cooled to 0°, 3.12 g (15 mM) of finely ground PCl_5 was added. The mixture was stirred for 1 hour at 0°, the excess of PCl_5 was filtered off, and the filtrate was evaporated in vacuum. The acid chloride obtained (in the form of an oil) was dissolved in 20 ml of dry ether and the solution was added (−20°, 1 hr) to a solution of 2.87 g (10 mM) of the aminoester (12) and 2.1 ml (15 mM) of triethylamine in 20 ml of dry ether; The mixture was stirred for a further 2 hr at 20°, washed with 1 N H_2SO_4, saturated $NaHCO_3$ solution, and water, dried with $MgSO_4$, and evaporated. The residue was chromatographed on neutral alumina in the benzene-ethyl acetate system (gradient elution). This gave 5.1 g (80%) of the tetradepsipeptide (13) in the form of an oil, $[\alpha]_D^{20}$ + 12° (c 1.5; benzene).

Found %: C 64.30; H 8.51; N 4.29. $C_{34}H_{54}O_9N_2$. Calculated %: C 64.33; H 8.57; N 4.41.

D-Alloisoleucyl-L-α-hydroxyisovaleryl-L-isoleucyl-L-α-hydroxyisovaleric acid hydrobromide (14). To a solution of 3.17 g (5 mM) of the protected tetradepsipeptide (13) in 5 ml of glacial acetic acid was added 20 ml of a 35% solution of hydrogen bromide in glacial acetic acid. The solution was left for 1 hour at 20° and evaporated to dryness; the residue was dissolved in water, and the aqueous solution was washed with ether and again evaporated to dryness in vacuum. This gave 2.14 g (80%) of the hydrobromide (14) in the form of an amorphous powder, $[\alpha]_D^{20}$ −30 °(c 0.7; alcohol).

Found %: Br 15.05; mol. wt. (titration with 0.01 N NaOH) 520.5. $C_{22}H_{41}O_7N_2Br$. Calculated %: Br 15.21; mol. wt. 525.48.

Angolide. A solution of 1.34 g (2.5 mmole) of the hydrobromide (14) in 10 ml of thionyl chloride was left for 30 min at 20°, the excess of thionyl chloride was distilled off in vacuum, dry toluene was added to the residue, and the solution was evaporated in vacuum. The resulting hydrochloride was dissolved in 200 ml of dry benzene and the solution was added in drops with stirring (20°, 10 hr) simultaneously with a solution of 0.84 ml (6 mM) of triethylamine in 200 ml of dry benzene to one liter of dry benzene. The mixture was left overnight at 20° and was evaporated in vacuum. The residue was dissolved in chloroform, the solution was washed with 1 N HCl, water, and saturated $NaHCO_3$ solution, then was dried with $MgSO_4$ and evaporated to dryness in vacuum. Recrystallization of the residue from alcohol gave 310 mg (28%) of angolide, mp 259-260°, $[\alpha]_D^{22}$ -83° (c 1.0; $CHCl_3$). (Cf. [4]). A mixture with natural angolide (mp 259.5-260°) gave no depression of the melting point.

SUMMARY

The structure of angolide — a depsipeptide produced by one of the strains of Pithomyces — has been established and its complete synthesis has been effected.

REFERENCES

1. W. S. Bertraud, [et al.]. J. Gen. Microbiol. 32, 385, 1963.
2. D. W. Russell. J. Chem. Soc., 753, 1962; D. W. Russell. Biophys. Acta. 45, 411, 1960; D. W. Russell, C. G. Macdonald, J. C. Shannon. Tetrah. Lett., 2759, 1964; E. Bishop, D. W. Russell. Biochem. J., 92, 19P, 1964.
3. M. M. Shemyakin [et al.] Tetrah. Lett., 19, 995, 1963; M. M. Shemyakin [et al.] Tetrah. Lett., 1921, 1963; A. A. Kiryüshkin, Yu. A. Ovchinnikov, M. M. Shemyakin. Tetrah. Lett., 3313, 1964.
4. D. W. Russell, V. Ward. Abstr. lst. Meeting Fed. European Biochem. Soc., London, 23-25th, March, 1964.
5. M. M. Shemyakin [et al.]. Tetrah., 19, 581, 1963.
6. M. M. Shemyakin [et al.] Tetrah. Lett., 1921, 1963.
7. N. S. Wulfson [et al.]. Lett., 951, 1964.

8. C. G. Macdonald, J. C. Shannon. Tetrah. Lett., 3113, 1964.

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