386. *Synthesis of Mycolipenic Acid.*

By D. J. Millin and N. Polgar.

The total synthesis of mycolipenic acid has been completed. (+)-2(*L*) : 4(*L*)-Dimethyldocosanoic acid * (I), an oxidation product of mycolipenic acid which has been previously synthesised,[1] was converted essentially by a route already described [2] for an optically inactive specimen into (+)-2 : 4(*L*) : 6(*L*)-trimethyltetracos-2-enoic acid (IV; R = H). The synthetic acid, regarded as the *trans*-isomer, is found to be identical with mycolipenic acid isolated from tubercle bacilli.

Previous work [1] resulted in the synthesis of (+)-2(*L*) : 4(*L*)-dimethyldocosanoic acid * (I) which proved identical with an oxidation product [3] of mycolipenic acid. It was, therefore, possible to use the oxidation product as a relay for the total synthesis of mycolipenic acid; the present paper describes this synthesis (for a preliminary report see ref. 4).

The procedure described earlier [2] for the synthesis of optically inactive 2 : 4 : 6-trimethyltetracos-2-enoic acid was followed in general. Reduction of (+)-2(*L*) : 4(*L*)-dimethyldocosanoic acid (I) with lithium aluminium hydride gave (−)-2(*L*) : 4(*L*)-dimethyldocosan-1-ol (II), $[\alpha]_D$ −6·0° (it is known [5] that an asymmetric centre adjacent to the carboxyl group is unaffected by this reduction). Condensation of the corresponding iodide, obtained *via* the toluene-*p*-sulphonyl ester, with the sodio-derivative of ethyl methylmalonate afforded by the usual successive stages (hydrolysis, decarboxylation) 2(*D*,*L*) : 4(*L*) : 6(*L*)-trimethyltetracosanoic acid (III). The resulting mixture of 2(*D*)- and 2(*L*)-diastereoisomers was used for the next stage; it is, however, of interest that the diastereoisomers were separable from each other by chromatography of their methyl esters over alumina. Bromination of the acid (III) (Hell–Volhard–Zelinsky method), followed by reaction of the α-bromo-acid bromide with methanol and dehydrobromination of the resulting α-bromo-ester by means of pyridine, gave (+)-[methyl 2 : 4(*L*) : 6(*L*)-trimethyltetracos-2-enoate] (IV; R = Me), $[\alpha]_D$ +16·4°, which from its method of preparation is regarded as the *trans*-isomer; its infrared absorption spectrum was in close agreement with that of methyl mycolipenate having $[\alpha]_D$ +16·8° (for isolation of the sample, see Experimental section). Hydrolysis of the synthetic ester afforded the corresponding acid (IV; R = H), m. p. 28°, $[\alpha]_D$ +19·3°, to be compared with m. p. 27° and $[\alpha]_D$ +19·3° for mycolipenic acid; determination of the mixed melting point and spectroscopic (ultraviolet and infrared) comparison supported their identity. The structure previously proposed [3] for mycolipenic acid is thus confirmed synthetically.

Cason and Sumrell [6] isolated from tubercle bacilli, among other products, a dextrorotatory αβ-unsaturated acid which they named "C_{27}-phthienoic acid." The physical data given [6,7] for this acid and its methyl ester are in close agreement with those found for

* The symbol *L* refers to the convention of Linstead, Lunt, and Weedon (*J.*, 1950, 3333).

[1] Fray and Polgar, *J.*, 1956, 2036.
[2] Bailey, Polgar, and Robinson, *J.*, 1953, 3031.
[3] Polgar and Robinson, *Chem. and Ind.*, 1951, 685; Polgar, *J.*, 1954, 1008.
[4] Millin and Polgar, *Proc. Chem. Soc.*, 1957, 122.
[5] Noyce and Denney, *J. Amer. Chem. Soc.*, 1950, **72**, 5743; see also ref. 1.
[6] Cason and Sumrell, *J. Biol. Chem.*, 1951, **192**, 405.
[7] Allen and Cason, *ibid.*, 1956, **220**, 407.

mycolipenic acid and methyl mycolipenate, and the infrared absorption spectrum [8] of methyl C_{27}-phthienoate does not appear to show any significant difference from that of

(I) $Me\cdot[CH_2]_{17}\cdot\overset{L}{C}HMe\cdot CH_2\cdot\overset{L}{C}HMe\cdot CO_2H$

(II) $Me\cdot[CH_2]_{17}\cdot\overset{L}{C}HMe\cdot CH_2\cdot\overset{L}{C}HMe\cdot CH_2\cdot OH$

(III) $Me\cdot[CH_2]_{17}\cdot CHMe\cdot CH_2\cdot CHMe\cdot CH_2\cdot\overset{DL}{C}HMe\cdot CO_2H$

(IV) $Me\cdot[CH_2]_{17}\cdot\overset{L}{C}HMe\cdot CH_2\cdot\overset{L}{C}HMe\cdot CH{:}CMe\cdot CO_2R$

methyl mycolipenate. However, in a recent note Cason, Urscheler, and Allen [9] express the view that C_{27}-phthienoic acid is not 2 : 4 : 6-trimethyltetracos-2-enoic acid: this view is mainly based on a study of a mixture of products arising on ozonisation of C_{27}-phthienoic acid, but there is no clear evidence on the nature of these ozonisation products, and none of them has been characterised by conversion into crystalline derivatives.

Experimental

Ultraviolet spectra were determined for EtOH solutions, and infrared spectra for natural films. M. p.s denoted (K) were observed on the Kofler block and are corrected; all other m. p.s were determined in a capillary tube and are uncorrected. Optical rotations were measured in a 1-dm. tube except where otherwise stated. Petrol refers to light petroleum, b. p. 40—60°. The alumina used for chromatography was acid-washed, and had activity II on Brockmann and Schodder's scale.[10]

(−)-2(L) : 4(L)-*Dimethyldocosan*-1-*ol* (II).—The starting material, (+)-2(*L*) : 4(*L*)-dimethyldocosanoic acid (I), was obtained by the procedure described earlier [3] except that (i) for the oxidation with potassium permanganate the methyl esters, b. p. 231—235°/2 mm. (resulting on fractionation of the mixture of esters as described in section " Isolation of Mycolipenic Acid "), were used after removal of straight-chain esters as urea complexes, and (ii) the acidic oxidation product was subjected to chromatography over silica. The acid (970 mg.; $[\alpha]_D$ +7·1°) in ether (15 c.c.) was added dropwise with stirring to a solution of lithium aluminium hydride (430 mg.) in ether (25 c.c.) during 15 min.; the mixture was then refluxed with stirring for 3 hr. Next day the excess of lithium aluminium hydride was decomposed by dropwise addition, with stirring, of moist ether and subsequently water. Ice-cold 10% sulphuric acid (40 c.c.) was then gradually added and the ethereal layer separated; the aqueous phase was extracted with ether, and the combined ethereal extracts were washed, successively, with water, 2% aqueous sodium hydrogen carbonate, and water, then dried ($MgSO_4$), and evaporated. The residue was chromatographed in petrol on alumina (90 g.; 12 × 3 cm.) prepared in petrol. Petrol–benzene (1 : 1) eluted (−)-2(L) : 4(L)-*dimethyldocosan*-1-*ol* as a white solid (700 mg.) which crystallised from petrol at −8° as rosettes, m. p. (K) 43—45°, $[\alpha]_D^{19}$ −6·0° (*c* 4·0 in ether) (Found: C, 81·0; H, 14·1. $C_{24}H_{50}O$ requires C, 81·3; H, 14·1%).

2(DL) : 4(L) : 6(L)-*Trimethyltetracosanoic Acid* (III).—Toluene-*p*-sulphonyl chloride (500 mg.) was added in three portions during 0·5 hr. to a solution of the above alcohol (620 mg.) in dry pyridine (3·5 c.c.) at 0°, the mixture being shaken. After a further 5 hr. at <15° with occasional shaking the mixture was set aside in a refrigerator. Next day it was poured into ice-cold water, acidified with dilute hydrochloric acid, and extracted with ether. The dried (K_2CO_3) extract was evaporated, affording the crude toluene-*p*-sulphonate as an oil, $[\alpha]_D^{16}$ +1·80° (*c* 3·8 in ether); the infrared spectrum indicated the absence of hydroxyl and showed bands at 1190 and 1355 cm.$^{-1}$ characteristic of $-O-SO_2-$. This ester was refluxed with a solution of anhydrous sodium iodide (360 mg.) in dry acetone (5 c.c.) for 13 hr. After a further 32 hr., water was added and the product extracted with ether. The extract was washed with aqueous sodium thiosulphate and then with water, dried (Na_2SO_4), and evaporated, yielding the iodide (720 mg.) as an oil, $[\alpha]_D^{17}$ +3·3° (*c* 5·4 in ether; *l* 0·5). Without further purification this iodide was refluxed with the sodio-derivative of ethyl methylmalonate (from 1·25 g. of ethyl methylmalonate, 80 mg. of sodium, and 5 c.c. of ethanol) for 14 hr., and the mixture then set aside overnight. After dilution with water and acidification with hydrochloric acid the product was isolated by means of ether, then refluxed with a solution of potassium hydroxide (2·5 g.)

[8] Cason, Freeman, and Sumrell, *J. Biol. Chem.*, 1951, **192**, 415.
[9] Cason, Urscheler, and Allen, *J. Org. Chem.*, 1957, **22**, 1284.
[10] Brockmann and Schodder, *Ber.*, 1941, **74**, 73.

in aqueous ethanol (1 : 2; 6 c.c.) for 7 hr. The resulting solution was diluted with water, acidified with hydrochloric acid, and the product, isolated by ether-extraction, decarboxylated by heating it at 160—175° (bath) for 2½ hr. The resulting acid (715 mg.) was esterified with ethereal diazomethane, and the methyl ester chromatographed in petrol on alumina (70 g.; 25 × 2 cm.) prepared in petrol. The following fractions were taken: (1) petrol (100 c.c.) (60 mg.); (2) petrol (400 c.c.) (45 mg.); (3) petrol (250 c.c.) (190 mg.); (4) petrol–benzene (9 : 1; 100 c.c.) (250 mg.); (5) petrol–benzene (9 : 1; 100 c.c.) (90 mg.); (6) petrol–benzene (9 : 1; 250 c.c.) (45 mg.); (7) benzene (300 c.c.) (trace).

Fraction (1) appeared to be essentially unchanged iodide. Fractions (3), (4), and (5), consisting of mixtures of the 2(*L*)- and 2(*D*)-diastereoisomers of the methyl ester with $[\alpha]_D^{20}$ +6·1°, +1·1°, and −1·02° (*c* 5—10 in $CHCl_3$), respectively, were combined and refluxed with 10% aqueous-ethanolic (3 : 7) potassium hydroxide (20 c.c.) for 2 hr. Acidification and ether-extraction gave the *acid* (480 mg.) as an oil which partially solidified (Found: C, 79·3; H, 12·9. $C_{27}H_{54}O_2$ requires C, 79·0; H, 13·1%).

trans-(+)-2 : 4(L) : 6(L)-*Trimethyltetracos-2-enoic Acid* (IV; R = H).—The preceding acid (480 mg.) was heated with bromine (slight excess) in the presence of red phosphorus (40 mg.) at 80—85° (bath) for 10 hr. in the manner previously described.[3] Next day anhydrous methanol (10 c.c.) was added and the mixture refluxed for 2·5 hr. Dilution with water and extraction with ether gave the crude bromo-ester which was then refluxed with dry pyridine (8 c.c.) for 19 hr. The product, isolated in the known manner, was passed in petrol over alumina. Elution with petrol (the first fractions being rejected) gave the *ester* (IV; R = Me) having $[\alpha]_D^{20}$ +16·4° (*c* 4·5 in $CHCl_3$), n_D^{16} 1·4632, $\lambda_{max.}$ 2170 Å (ε 13,600). This ester and methyl mycolipenate (see below) had infrared max. at 2924, 2857, 1721, 1650, 1460, 1435, 1376, 1307, 1271, 1218, 1189, 1149, 1098, 749, and 718 $cm.^{-1}$. Hydrolysis of the synthetic ester by refluxing it with 5% methanolic potassium hydroxide, followed by acidification with hydrochloric acid, gave the *acid* which crystallised from acetone at −8° as microcrystalline rosettes, m. p. 28°, $[\alpha]_D^{21}$ +19·3° (*c* 3 in $CHCl_3$), $\lambda_{max.}$ 2160 Å (ε 13,900) (Found: C, 79·1; H, 12·5. $C_{27}H_{52}O_2$ requires C, 79·4; H, 12·8%). The acid did not depress the m. p. of natural mycolipenic acid. Its *quinine salt* crystallised from acetone at −8° as needles, m. p. (K) 87—88° (Found, after drying at 65° under vacuum for 48 hr.: C, 77·2; H, 10·4; N, 3·5. $C_{47}H_{76}O_4N_2$ requires C, 76·9; H, 10·45; N, 3·8%); upon admixture with the quinine salt (m. p. 85—86°) prepared from the naturally occurring acid the m. p. was 85—87°.

Isolation of Mycolipenic Acid.—The procedure described in an earlier paper [11] was employed with the following modifications. The methyl esters, fraction (b), obtained from the acids resulting on partial hydrolysis of the extracts (A) and (B), were chromatographed in petrol over silica, and the material eluted by petrol was fractionated through a Podbielniak column (75 cm.) of the concentric-tube type. The fraction, b. p. 230—230·5°/2 mm., $[\alpha]_D^{18}$ +9·82° (*c* 9·4 in ether; *l* 0·5) (0·47 g.), was dissolved in petrol (4 c.c.); urea (1·2 g.), moistened with a few drops of methanol, was added. The mixture was kept overnight; the solid was then filtered off and washed with petrol. The filtrate and washings were washed with water, dried (Na_2SO_4), and evaporated, affording an oil (0·38 g.) having $[\alpha]_D^{15}$ +11·55° (*c* 7·6 in ether; *l* 0·5) A further treatment with urea as above (the straight-chain ester removed as a urea complex was found to be essentially methyl hexacosanoate) left branched-chain esters (0·35 g.) with $[\alpha]_D^{15}$ +11·6° (in ether), +11·8° (in $CHCl_3$) (*c* 7·0; *l* 0·5). Repeated chromatography of this product in petrol over alumina, the first fractions eluted by petrol being rejected each time, gave methyl mycolipenate as an oil having $[\alpha]_D^{23}$ +16·8° (*c* 4·0 in $CHCl_3$), n_D^{16} 1·4633, $\lambda_{max.}$ 2170 Å (ε 13,800) {Cason and Sumrell [6] record $[\alpha]_D^{25}$ +14·7°, n_D^{25} 1·4600, $\lambda_{max.}$ 2180 Å (ε 12,230) for methyl C_{27}-phthienoate}. Hydrolysis of this ester with 5% methanolic potassium hydroxide afforded mycolipenic acid which crystallised from acetone at −8° as microcrystalline rosettes, m. p. 27°, $[\alpha]_D^{21}$ +19·3° (*c* 3 in $CHCl_3$), $\lambda_{max.}$ 2160 Å (ε 14,100). Its *quinine salt* crystallised from acetone at −8° as needles, m. p. (K) 85—86° (Found: C, 76·8; H, 10·6. $C_{47}H_{76}O_4N_2$ requires C, 76·9; H, 10·45%). Cason and Sumrell [6] and Allen and Cason [7] report several polymorphic forms for C_{27}-phthienoic acid, samples obtainable by low-temperature crystallisation from acetone having m. p. 26—27°; the last-named authors record for the acid $[\alpha]_D^{25}$ +19·6° ($CHCl_3$) and $\lambda_{max.}$ 2180 Å (ε 13,200 in heptane).

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DYSON PERRINS LABORATORY, OXFORD UNIVERSITY. [*Received, January 14th,* 1958.]

[11] Chanley and Polgar, *J.*, 1954, 1003.