766. *The Chemistry of Fungi. Part XLIX.*[1] *Aliphatic Amides from Ergot*

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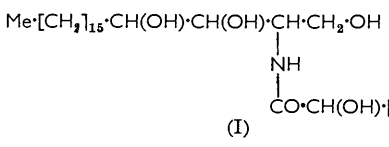
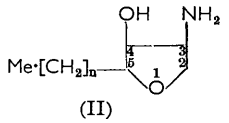
A new cerebrin like material, ergocerebrin, $C_{42}H_{83}NO_4$ (IV; $n = 21$), and a known cerebrin (V) have been isolated from ergot residues.

Various relatively ill-characterised aliphatic secondary amides, the cerebrins, analogous to the ceramides [2] of animal origin, have been isolated from fungi. The first cerebrin was obtained in 1911 by Zellner [3] from *Amanita muscaria*, whilst from yeast Reindel,[4] in 1930, obtained a cerebrin, $C_{46}H_{93}NO_5$, which was converted into an anhydrocerebrin, $C_{46}H_{91}NO_4$, by prolonged boiling with hot methanolic mineral acid. Hydrolysis of yeast cerebrin gave 2-hydroxyhexacosanoic acid and a base, $C_{20}H_{43}NO_3$, whilst hydrolysis of anhydrocerebrin furnished the same acid but an anhydro-base, $C_{20}H_{41}NO_2$ (cf. Prostenik *et al.*[5]). On the basis of these and other results yeast cerebrin and the anhydro-base were formulated as (I) and (II; $n = 15$), respectively. Cerebrin (I) has also been isolated [6] from the mycelium of *Aspergillus sydowi*. In 1952, Oda [7] obtained a cerebrin, $C_{42}H_{85}NO_5$, and an oxycerebrin, $C_{42}H_{85}NO_6$, from the mycelium of *Penicillium notatum*.

As part of our comprehensive investigations [1,8,9] into the non-alkaloidal constituents of ergot we have examined the residues remaining after removal of the phenolic pigments and have obtained a mixture of closely related substances. The principal of these, which we propose to call ergocerebrin, $C_{42}H_{83}NO_4$, probably has the structure (IV; $n = 21$).



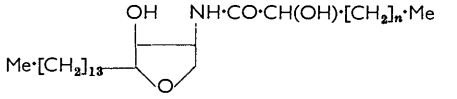
(I)



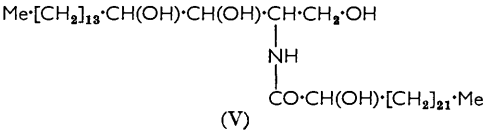
(II)

$Me{\cdot}[CH_2]_{13}{\cdot}CO{\cdot}CO_2Me$

(III)



(IV)



(V)

Although our ergocerebrin was obviously contaminated with smaller amounts of very closely related compounds, alkaline hydrolysis gave a base which had all the characteristics of a pure substance; thus, *inter alia*, its melting point and the melting point of its derivatives were sharp, and on thin-layer chromatography under a variety of conditions it ran as a single spot. Elemental analysis of the base and its derivatives agree with the formula $C_{18}H_{37}NO_2$. The base has no significant ultraviolet absorption but the infrared spectrum (in chloroform) has $\nu_{max.}$ 3100 (bonded OH or NH), 2700 (strongly bonded OH), and 912 and 1078 (tetrahydrofuran) cm.$^{-1}$. The base yields an *NO*-diacetyl derivative, $C_{22}H_{41}NO_4$, the infrared spectrum of which is devoid of hydroxyl absorption; the n.m.r.

[1] Part XLVIII, preceding Paper.
[2] H. Deuel, "The Lipids," Interscience, New York, 1951, vol. I, p. 460, 491.
[3] P. Zellner, *Monatsh.*, 1911, **32**, 133.
[4] F. Reindel, *Annalen*, 1930, **480**, 76.
[5] M. Prostenik, B. Majhofer-Orescanin, M. Munk-Weinert, and B. Ries-Lesic, *Croat. Chem. Acta*, 1960, **32**, 11.
[6] N. Bohonos and W. H. Peterson, *J. Biol. Chem.*, 1943, **149**, 295.
[7] T. Oda, *J. Pharm. Soc. Japan*, 1952, **72**, 136, 139, 142.
[8] J. W. ApSimon, J. A. Corran, N. G. Creasey, K. Y. Sim, and W. B. Whalley, *J.*, 1965, 4130.
[9] J. W. ApSimon, J. A. Corran, N. G. Creasey, W. Marlow, W. B. Whalley, and (in part) K. Y. Sim, *J.*, 1965, 4144.

spectrum of this acetate shows signals at τ 9·1 (distorted triplet? terminal $CH_2 \cdot CH_3$, J 6 c./sec., 3 protons), 7·88 ($O \cdot CO \cdot CH_3$, singlet, 3 protons), and 8·00 ($NH \cdot CO \cdot CH_3$, singlet, 3 protons). These results clearly indicate (a) the presence of only one terminal methyl residue, (b) that the second oxygen atom is inert (ether linkage) and hence an " anhydro-base " structure of type (II; $n = 13$). This is substantiated by the results of oxidation with periodate, when one molecular proportion of oxidising agent was consumed rapidly. Chemical methods did not conclusively establish the nature of the amine residue but the n.m.r. spectrum of the base included a diffuse signal centred at τ 7·87 (3 protons); since this disappeared upon deuteration it is ascribed to the OH and NH_2 residues, and hence the amino-group must be primary. Information concerning the length of the side-chain was afforded by oxidation with chromic oxide, when two main fractions were obtained. The principal component (*ca.* 85%) of the first was identified (as the methyl ester) as pentadecanoic acid by gas chromatography and by mass spectrometry. The mass spectrum of the second fraction from the oxidation gave a strong fragment peak at mass number 225. This is compatible with the presence of the ester (III), and thus strongly indicative of substitution (by OH or NH_2) at C-4 [formula (II)]. Since the hydroxyl group is not acidic it cannot be located at C-2. Our anhydro-base is therefore probably represented by one of the diastereoisomers of formula (II; $n = 13$). The relative positions of the hydroxyl and amino-residues have not been unequivocally defined but are in accord with general biogenetic considerations and previous structural allocations within this group.

Although direct comparison has not been possible the base (II; $n = 13$) from ergocerebrin may be identical with the phytosphingosine anhydrobase isolated from corn phosphatides.[10]

In contrast to the base, the acid fraction from the hydrolysis of ergocerebrin was not homogeneous. The major component was cerebronic acid, together with minor amounts of closely related homologues. Thus, the mass spectrum of the methyl ester indicated the following approximate composition: methyl α-hydroxytetracosanoate (cerebronate) 70%, methyl α-hydroxytricosanoate 15%, and methyl α-hydroxydicosanoate 15%. The strong peak at mass number 90 clearly showed that the esters contained an α-hydroxyl group. Oxidation of this cerebronic acid fraction gave impure tricosanoic acid, which was identified (as the methyl ester) by both gas chromatography and mass spectroscopy. Both techniques showed the presence of about 70% of methyl tricosanoate, whilst mass spectroscopy also showed the presence of about 20% of the methyl esters of di- and monocosanoic acids, and the absence of α-hydroxy-esters. Although methylation of cerebronic acid with diazomethane proceeded normally, the action of dimethyl sulphate in boiling acetone containing potassium carbonate gave rise to a neutral product, devoid of methoxyl groups, having the properties of the corresponding lactide.

Whilst ergocerebrin is too sparingly soluble for a satisfactory determination of the n.m.r. spectrum, the di-*O*-acetate gave a spectrum which showed signals at τ 7·8 ($O \cdot CO \cdot CH_3$, singlet, 3 protons), and 7·9 ($O \cdot CO \cdot CH_3$, singlet, 3 protons). The infrared spectrum of this di-*O*-acetate was devoid of hydroxyl absorption. Consequently it follows that the anhydro-base structure is present in ergocerebrin, which may be formulated as (IV; $n = 21$). Ergocerebrin is accompanied by minor amounts of analogues including (IV; $n = 20$) and (IV; $n = 19$). The presence of aliphatic acid residues containing an odd number of carbon atoms may be noted.

Since the base appears to be homogeneous and the acidic fraction contains *ca.* 70% of cerebronic acid, our mixture of amides must contain at least 85% of ergocerebrin (IV; $n = 21$).

Previous workers in this field have recognised that the anhydro-bases isolated by them have almost certainly been artifacts, produced by the cyclisation of the basic fragment in, *e.g.*, (I) to a tetrahydrofuran derivative by the prolonged action of the hot mineral acid used for hydrolysis. Since, to the best of our knowledge, ergocerebrin has experienced

[10] P. W. O'Connell and S. H. Tsien, *Arch. Biochem. Biophys.*, 1959, **80**, 289.

only minimal exposure to low concentrations of mineral acids under very mild conditions which do not effect the cyclisation of the open-chain precursor (V) (cf. ref. 10), it appears probable that ergocerebrin is a true natural product and not an artifact.

After removal of ergocerebrin by benzene extraction the ergot residues were extracted with chloroform to give a quantity of the cerebrin (V) which was apparently identical with that obtained by Oda.[7]

Experimental

Infrared spectra were determined in Nujol using Infracord 137 and 237 spectrometers. N.m.r. spectra were determined in deuteriochloroform on a Varian A60 spectrometer by Miss J. Lovenack.

Light petroleum refers to the fraction of b. p. 60—80°.

Ergocerebrin.—The fraction insoluble in acetone of the ergot residues (3 kg.) remaining after isolation of the pigments was dried, powdered (if not too sticky), and defatted by extraction in a Soxhlet apparatus with boiling light petroleum for 48 hr., followed by extraction for 5 days with boiling benzene. The benzene extract was concentrated to 25% of the original volume. On cooling, a semicrystalline material separated. Repeated purification from ethanol (charcoal) gave *ergocerebrin* (15—30 g.) in clusters of fibrous needles, m. p. 106—107°, $[\alpha]_D^{20}$ +16·3° (*c* 0·57 in tetrachloroethane) (Found: C, 75·2, 75·4, 75·6; H, 12·1, 11·5, 12·1; N, 2·3, 2·2, 2·1; O, 10·0, 10·4, 10·3. $C_{42}H_{83}NO_4$ requires C, 75·8; H, 12·5; N, 2·1; O, 9·6%). This material is very sparingly soluble in the usual organic solvents, but has the greatest solubility in chlorinated solvents and pyridine. Evaporation of the light-petroleum extract furnished a dark syrup which was diluted with acetone. After several months ergocerebrin (*ca.* 0·5 g.) separated.

Prepared by the acetic anhydride–pyridine method, the *di*-O-*acetyl derivative* separated from ethanol in clusters of needles, m. p. 75°, $[\alpha]_D^{20}$ −7° (*c* 2·5 in chloroform) (Found: C, 73·6; H, 11·4; N, 1·8. $C_{46}H_{87}NO_6$ requires C, 73·7; H, 11·7; N, 1·9%).

The *di*-p-*nitrobenzoate* formed needles, m. p. 95° (from ethanol) (Found: C, 70·1; H, 9·3; N, 4·3; O, 16·5. $C_{56}H_{89}N_3O_{10}$ requires C, 69·8; H, 9·2; N, 4·3; O, 16·7%).

Hydrolysis of ergocerebrin (5·6 g.) with boiling 10% ethanolic potassium hydroxide (150 ml.) was complete in 6 hr., and the solution was concentrated (100 ml.). Next day the crystalline deposit was collected, washed with water, and exhaustively extracted with ether. Evaporation of the ether furnished the anhydro-base.

The Anhydro-base.—This base separated from methanol in plates, m. p. 105°, or from light petroleum in needles, m. p. 105° (lit.,[10] 105—106°), $[\alpha]_D^{20}$ +20·1° (*c* 1·12 in ethanol) [Found: C, 72·3; H, 12·3; N, 4·4%; *M* (Rast), 302. Calc. for $C_{18}H_{37}NO_2$: C, 72·2; H, 12·5; N, 4·7%; *M*, 299). This base ran as one spot under various conditions; *e.g.*, on Silica Gel G plates using the solvent system chloroform–methanol–2N-ammonium hydroxide (40 : 10 : 1). The spots were developed with ninhydrin or potassium permanganate.

The NO-*diacetate* formed microcrystals, m. p. 79° (from light petroleum), $[\alpha]_D^{20}$ −21·9° (*c* 0·5 in chloroform) (Found: C, 68·6; H, 10·7; N, 4·2. $C_{22}H_{41}NO_4$ requires C, 68·9; H, 10·8; N, 3·7%).

Prepared from the base (0·25 g.), benzoyl chloride (1·5 g.), and pyridine (4 ml.) during 12 hr., the NO-*dibenzoate* formed plates (0·24 g.), m. p. 122·5—123·5° (from ethanol), $[\alpha]_D^{20}$ −58·4° (*c* 0·4 in chloroform) (Found: C, 75·8; H, 8·6; N, 2·8. $C_{32}H_{45}NO_4$ requires C, 75·8; H, 8·9; N, 2·9%). This dibenzoate (0·15 g.) was refluxed for 1 hr., with 0·5N-methanolic potassium hydroxide (20 ml.). The cooled hydrolysate was extracted with a 1 : 1 mixture of methylene chloride–water. The organic layer was dried and evaporated, to yield the *N*-benzoate (0·13 g.) as plates, m. p. 108—109° [from benzene–light petroleum (1 : 3)], $[\alpha]_D^{22}$ −59° (*c* 1·0 in chloroform) (Found: C, 74·0; H, 10·1; N, 3·3. Calc. for $C_{25}H_{41}NO_3$: C, 74·4; H, 10·2; N, 3·5%). O'Connell and Tsien [10] record m. p. 108—109° for a specimen prepared by an alternative method.

Oxidation of the Anhydro-base.—(*a*) A solution of the base (1 g.) in acetic acid (200 ml.) containing chromic oxide (20 g.) was kept at 70° for 1 hr. Next day the mixture was diluted with water (1·5 l.) and extracted with ether. The crude acidic product was esterified by treatment with excess of ethereal diazomethane and distilled to yield (i) a mobile colourless liquid (*ca.* 0·4 g.) (crude methyl pentadecanoate), b. p. 120—130°/0·1 mm., and (ii) a low-melting solid (*ca.* 0·2 g.), b. p. 140—160°/0·1 mm.

Analysis of fraction (i) using a 1-m. column with Apiezon L as stationary phase and a hydrogen flow rate of 110 ml./min., at 235°, gave a main peak having a retention time of 9 min. (86%). Methyl myristate, palmitate, and stearate gave comparative retention times of 6, 12, and 29 min., respectively, from which data fraction (i) was identified as methyl pentadecanoate. Fraction (ii) was too involatile to be analysed by gas chromatography.

(*b*) A solution of the base (3·0 mg., 1 × 10^{-5} mole) in methanol (1 ml.) was rapidly mixed with 0·015M-sodium periodate (1 ml., 1·5 mole of IO_4) in methanol–water (98 : 2) and transferred to an Agla micrometer syringe. Aliquot portions (0·2 ml.) were removed at intervals of 4, 9, 36, 95, and 210 min., diluted to 25 ml. with distilled water, and the optical density measured in 1-cm. cells at 223 mμ (using a distilled-water blank). The optical densities of the specimens were 0·37, 0·33, 0·28, 0·25, and 0·18. Since the optical density of the 0·015M-periodate solution at 223 mμ is 0·60 and the equivalent for iodate ion is 0·07 it follows that the base consumed 1 mol. of periodate in *ca.* 1½ hr. Further slow consumption of periodate occurred over a longer period of time (presumably owing to oxidation of the initial reaction products).

The Acid from the Hydrolysis of Ergocerebrin.—The crude acid was purified by repeated crystallisation from light petroleum containing 7% of acetic acid, to yield impure cerebronic acid, needles, m. p. 98·5—99°, $[\alpha]_D^{19}$ +4° (*c* 2·0 in pyridine) (Found: C, 74·6, 74·7; H, 12·5, 12·8%; Equiv., 364. Calc. for $C_{24}H_{48}O_3$: C, 74·9; H, 12·6%; Equiv., 384). Esterification with diazomethane gave impure methyl cerebronate, needles, m. p. 71° (from methanol), $[\alpha]_D^{20}$ +1·2° (*c* 1·12 in $CHCl_3$) [Found: C, 75·2; H, 12·6; OMe, 8·0. Calc. for $C_{24}H_{47}O_2(OMe)$: C, 75·3; H, 12·6; OMe, 7·8%].

A solution of this impure cerebroic acid (1 g.) in acetone (150 ml.) and water (50 ml.) containing potassium permanganate (2 g.) was refluxed for ½ hr. The cooled mixture was clarified with sulphur dioxide and the acetone removed *in vacuo*; a crystalline product separated. Purification from aqueous acetone gave impure tricosanoic acid (*ca.* 0·6 g.), prisms, m. p. 76° (Found: C, 78·0; H, 13·2. Calc. for $C_{23}H_{46}O_2$: C, 77·9; H, 13·1%).

Impure methyl tricosanoate formed plates, m. p. 54° (from acetone) [Found: C, 78·3; H, 13·3; OMe, 8·7. Calc. for $C_{23}H_{45}O(OMe)$: C, 78·2; H, 13·1; OMe, 8·4%]. Analysis of this ester, on a polyethylene glycol column (1 m. length) at 200° using argon at 7 p.s.i. showed that it contained 75—80% of methyl tricosanoate.

Methylation of impure cerebronic acid (0·5 g.) in boiling acetone (40 ml.) containing potassium carbonate (5 g.) and dimethyl sulphate (3 ml.) during 3 hr., gave the *lactide*, prisms, m. p. 68° (from light petroleum) (Found: C, 77·8; H, 12·8; OMe, 0. $C_{48}H_{92}O_4$ requires C, 78·6; H, 12·7%).

A sample of the impure methyl cerebronate was fully methylated by the action of boiling methyl iodide and silver oxide until a sample no longer exhibited hydroxyl absorption in the infrared spectrum. The product was analysed on a 2-m. silicone grease column at 247° with a hydrogen flow rate of 220 ml./min.; samples (5 ml.) of ester dissolved in methylcyclohexane were injected on to the column. The results indicated an 89% content of methyl 2-*O*-methylcerebronate having a retention time of 140 min.

Isolation of Cerebrin (V) *from Ergot* (with K. Y. Sim).—The residues remaining from the benzene extraction of ergochrysin were exhaustively extracted with hot chloroform (4 days) in a Soxhlet apparatus. Concentration of the extract gave a black viscous syrup which was purified by washing with acetone; a brown friable solid remained. Repeated crystallisation from acetone gave cerebrin, m. p. 147—148°, small prisms, $[\alpha]_D$ +10·4° (*c* 1·4 in pyridine) (Found: C, 73·3; H, 12·4; N, 2·1. Calc. for $C_{42}H_{85}NO_5$: C, 73·8; H, 12·5; N, 2·1%). This compound had the same m. p., mixed m. p., and infrared spectrum as a specimen of cerebrin isolated by Oda.[7]

Hydrolysis of cerebrin (1·5 g.) with boiling 10% ethanolic potassium hydroxide (120 ml.) during 5 hr. gave an acid (0·3 g.) which formed needles, m. p. 102—104° (from acetone or methanol), and appeared to be identical with the cerebronic acid fraction of ergocerebrin $[\alpha]_D^{20}$ +2·8° (*c* 7·0 in pyridine) (Found: C, 75·5; H, 12·5. Calc. for $C_{24}H_{48}O_3$: C, 74·9; H, 12·6%).

The base was most readily purified as the acetone complex which formed prisms, m. p. 108—109° (lit.,[7] 110°) (Found: C, 70·5; H, 11·7; N, 4·1. Calc. for $C_{21}H_{45}NO_3$: C, 70·1; H, 12·5; N, 3·9%).

We thank Messrs. Burroughs Wellcome for the supply of ergot residues. One of us (A. J. H.) is indebted to the Glaxo Group Ltd. for the award of a Post-Doctoral Fellowship. Analyses

were by Mr. G. Crouch and his associates. We are indebted to Dr. J. N. T. Gilbert for assistance with the gas chromatography. Dr. T. Yamakawa, Tokyo, supplied a sample of the cerebrin isolated by the late Dr. T. Oda. We are grateful to Dr. A. J. Bowen, Shell Research Ltd., for the determination of the mass spectra (in an MS.9 instrument) and for assistance in their interpretation.

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