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LONG CHAIN α,ω-DICARBOXYLIC ACIDS FROM THE SPORES OF *EQUISETUM TELMATEIA* AND *E. ARVENSE**

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Abstract—Ether extraction of spores of *Equisetum telmateia* and *E. arvense* gives up to 1% triacontanedioic acid (equisetolic acid) contaminated with a small proportion of octacosanedioic acid. The dimethyl ester of the C_{30}-diacid has been isolated in a pure state by GLC and its identity confirmed by comparison with a sample prepared by anodic synthesis.

INTRODUCTION

SPORES and pollen are not only formed in large quantity, but they are morphologically and chemically perhaps the most enduring objects produced in the plant kingdom. Fossil spores are preserved in peat and various sediments, in lignite, coal, and, possibly, even in Precambrian shales.[1] Until recently the chemistry of spores and pollen has attracted rather little sustained attention. The *Equisetaceae* have an especial interest since the 25 or so species of *Equisetum* that remain are the evolutionary residue of what was, on the fossil evidence, a large and widespread family. We have examined the spores of two of the commonest species, *E. telmateia* Ehrh. (*E. maximum* Lam, the Giant Horsetail) and *E. arvense* (the Common Horsetail).

In 1949, Sosa[2] reported that spores of *E. telmateia* gave, on ether extraction, a colourless saturated acid (0·4%, m.p. 127·5°) which was called equisetolic acid. The potassium salt, a monoacetyl derivative (m.p. 119°), and the dimethyl ester (m.p. 83–83·5°) were described. Sosa concluded that equisetolic acid was a monohydroxyheptatriacontanedioic acid ($HOOC–C_{35}H_{69}(OH)–COOH$). Further extraction of the spores with ethanol afforded two flavonolic glycosides, equisporoside and equisporonoside, which were not fully identified.[3]

Equisetolic acid, as formulated by Sosa, appeared to be a most unusual lipid. Natural dicarboxylic acids of this chain length had not previously been reported and, further, it appeared possible that such a substance might, if produced in similar quantity by the now extinct but once flourishing relatives of *Equisetum*, have played some part in the formation of natural hydrocarbon deposits. It was therefore determined to isolate equisetolic acid and establish its constitution in detail.

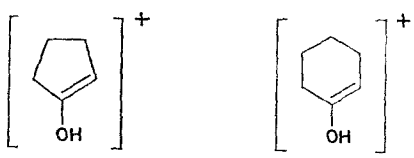
* Preliminary communication: K. R. ADAMS, R. BONNETT, J. HALL and J. P. KUTNEY, *Chem. Commun.* 456 (1969).

[1] L. R. WILSON, *J. Sediment. Petrol.* **16**, 110 (1946); G. ERDTMAN, *Advan. Botan. Res.* **1**, 149 (1963).
[2] A. SOSA, *Bull. Soc. Chim. Biol.* **31**, 57 (1949); *Ann. Sci. Nat. Bot.* **10**, 201 (1949).
[3] Further work on these glycosides has been undertaken by J. Kutney and J. Hall (University of British Columbia). We thank Dr. Kutney for informing us of these results in advance of publication. See also T. NAKABAYASHI, *J. Agri. Chem. Soc. Japan* **32**, 436 (1958).

RESULTS AND DISCUSSION

Air-dried spores of *E. arvense* were subjected to a preliminary washing with dichloromethane to remove the more soluble lipids.[4] Continuous ether extraction then yielded a white solid (up to 1% of the dry weight of the spores) which on recrystallization gave a colourless crystalline substance (m.p. 126–127°) which was presumed to be the material described by Sosa. This substance was, indeed, acidic and treatment with diazomethane gave the dimethyl ester, m.p. 83–84°. The IR spectrum of the ester showed the expected carbonyl absorption (1746 cm^{-1}) but, in Nujol, no band attributable to the hydroxyl function. The infra-red of the disodium salt showed only carboxylate absorption (1556 cm^{-1}) in the double bond region, and again no hydroxyl absorption was apparent. Sosa's main evidence for the hydroxyl function had been the acetyl derivative mentioned previously. However, when the acetylation of equisetolic acid was repeated only starting material was recovered,[5] and it was concluded that an alcoholic hydroxyl group was not present in equisetolic acid.

Total analysis of dimethyl equisetolate indicated, on the basis of an O_4 formulation, the molecular formula $C_{32}H_{62}O_4$, and this was confirmed by an accurate measurement of the molecular ion (m/e = 510·465; $C_{32}H_{62}O_4$ requires 510·465). The fragmentation pattern (Fig. 1) showed features expected for the ester of a long chain dicarboxylic acid[6,7] and corresponded in detail to the spectrum expected for dimethyl triacontanedioate. The series of ions at $m/e = 84 + 14n$ are not found in the spectra of esters of monocarboxylic acids, and have been attributed[7] to cyclic ions of the type:



m/e = 84 m/e = 98

the larger ions of the series presumably corresponding principally to alkyl-substituted six-membered rings.

The IR spectrum of dimethyl equisetolate showed a prominent 'fatty acid band progression' in the 1200–1350 cm^{-1} region. The NMR spectrum was remarkably simple, and showed signals at τ 6·39 (s, OMe) and 7·75 (m, $—CH_2CO$), the remaining (52) methylene protons appearing as a single broad peak at τ 8·73.

That dimethyl equisetolate was indeed dimethyl triacontanedioate ($MeOOC(CH_2)_{28}COOMe$) was confirmed by comparison with a synthetic sample prepared from ethyl hydrogen azelate by a two-fold anodic coupling procedure.[8] The overall yield (from ethyl hydrogen azelate) was 12%. The mass and NMR spectra of natural and synthetic samples were identical. The IR spectra were identical apart from a small discrepancy (an inversion in the intensities of the bands at 1305 and 1325 cm^{-1}) in the band progression, and this discrepancy was removed when samples purified by GLC were compared.

[4] This fraction, which contained polar material as well as hydrocarbons and wax esters, is still under investigation.

[5] It is inferred that the 'acetyl' derivative, m.p. 119°, described by Sosa was in fact an impure sample of equisetolic acid.

[6] R. RYHAGE and E. STENHAGEN, *Arkiv. Kemi.* **14,** 497 (1959).

[7] R. RYHAGE and E. STENHAGEN, *Arkiv. Kemi.* **23,** 167 (1965).

[8] cf. D A. FAIRWEATHER, *Proy. Roy. Soc. Edinburgh* **45,** 283 (1925).

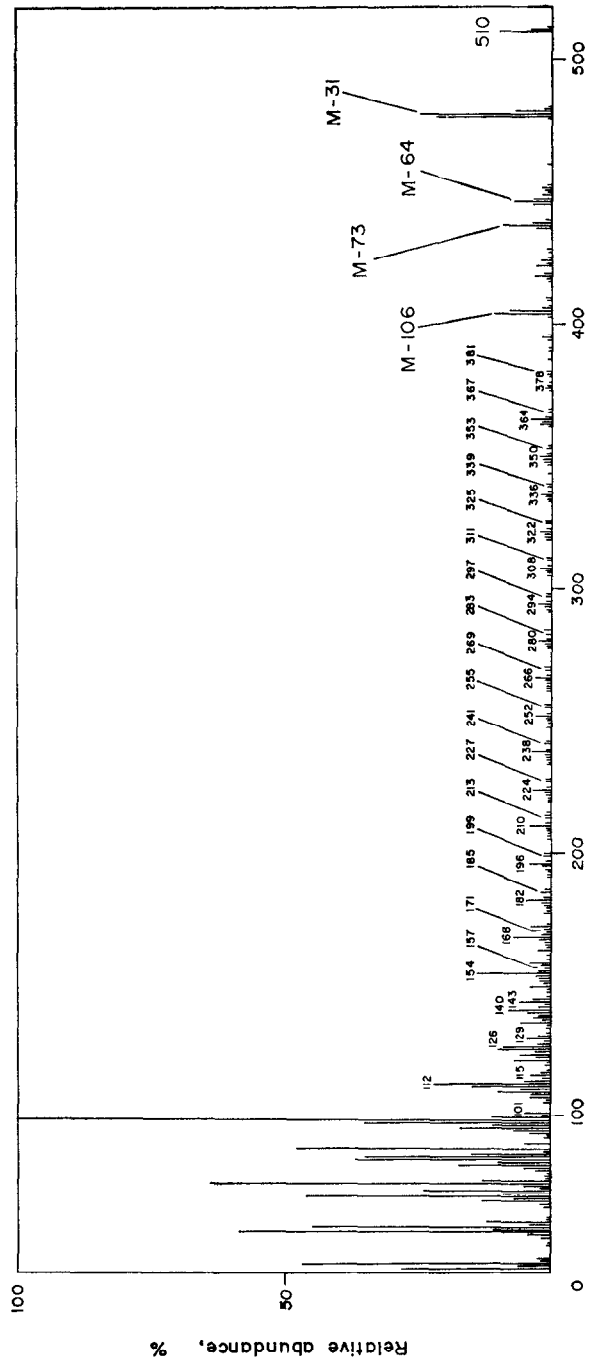


FIG. 1. MASS SPECTRUM OF DIMETHYL EQUISETOLATE.

The possibility that homologues of the C_{30}-diacid were present was examined by direct GLC of crude ester preparations using a deactivated ('silanized') support (see Experimental). This procedure indicated that the crude ester from *E. arvense* contained 97% dimethyl equisetolate, with trace amounts (see Table 1) of components which, on the basis of a log retention time/chain length plot, were the methyl esters of the C_{28}-, C_{24}- and C_{22}-dicarboxylic acids. The crude ester from *E. telmateia* contained 91% dimethyl equisetolate, and an appreciable proportion (4·6%) of the C_{28}-component. A small amount of the latter was isolated by preparative GLC: mass spectrometry then confirmed that this substance was dimethyl octacosanedioate on the basis of both the fragmentation pattern and the accurately measured molecular ion.

TABLE 1. COMPOSITION OF CRUDE DIACID FRACTIONS FROM EQUISETUM SPORES* MEASURED BY GLC OF CORRESPONDING DIMETHYL ESTERS

	Relative abundances of $\alpha\omega$-diacids			
	C_{22}	C_{24}	C_{28}	C_{30}
E. arvense	1 1	0·5	1·7	97
E telmateia†	2 3	1·5	4 6	91

* Chain lengths were assigned on the basis of a log retention time/chain length plot, assuming C_{30} for dimethyl equisetolate ($t_R \approx 53$ min).

† A small peak (*ca.* 1·5%) at an apparent chain length of 31·3 was also observed.

It is concluded that Sosa's formulation of equisetolic acid is incorrect. This substance is reformulated as triacontanedioic acid, which is accompanied in the spores by a small amount (*ca.* 5% in *E. telmateia*) of octacosanedioic acid, as well as by traces of other α,ω-diacids. Neither the C_{30}- nor the C_{28}-dicarboxylic acid appears previously to have been recognized in Nature. Japan wax is a well-known source of long-chain dicarboxylic acids and contains hexacosanedioic acid,[9] but higher homologues do not appear to have been reported. The structurally related 30-hydroxytriacontanoic acid has been isolated[10] from Carnauba wax, however.

Examination at low power magnification of the spores before and after extraction showed that they had not been ruptured, and that the elaters were apparently intact. This, together with the observation that little chlorophyll was extracted from the spores, might be taken to suggest that the equisetolic acid is located at or near the surface. Some support for this suggestion was provided during an examination of the spores at high magnification (Plate I).[11] The upper photographs (a and b) show a whole spore before and after the extraction. It is evident that the ribbon-like processes on the surface (shown under higher magnification in c and d) have been removed or diminished during the extraction process. Dr. Roger Ellis, whom we thank for these photomicrographs, informs us that, after extraction, the spores were much more susceptible to damage by the electron beam, which again accords with the suggestion that material has been removed from the spore wall.

[9] J. A. LAMBERTON, *Australian J. Chem.* **14**, 323 (1961).
[10] K. E. MURRAY and R. SCHOENFELD, *Australian J. Chem.* **8**, 437 (1955).
[11] The use of the 'Stereoscan' microscope (Cambridge Scientific Instruments Ltd.) is acknowledged.

The function of equisetolic acid is not clear. Presumably, like other superficial lipids in the plant kingdom, it serves as a protective covering, but its chemical structure (non-polar chain with two very polar end-groups) is rather atypical of epicuticular lipids, which are, indeed, often hydrocarbons or waxes.[12] Equisetolic acid is a remarkable natural lipid in that it occurs in the spores cited (i) in the free acid form, (ii) at a relatively high concentration, and (iii) with little contamination from homologues. Possibly it has a more explicit biological function, and it is tempting to suggest that it is involved in some way in the dehiscense process during which, in damp air,[13] the elaters uncurl from the spore body.

EXPERIMENTAL

General

M ps are uncorrected, and were determined in capillary tubes. All solvents were redistilled. IR spectra were determined in Nujol mulls or KBr discs using a Perkin–Elmer 257 or 225 grating instrument: NMR spectra were recorded on a Varian A60 spectrometer using tetramethylsilane as internal reference: mass spectra were recorded using the MS 902 (A.E.I. Ltd.) instrument, the samples being introduced by direct insertion. Elemental analyses were by Alfred Bernhardt.

Plant Material

Cones from *Equisetum arvense* and *E. telmateia* were collected from Lulu Island, Vancouver, B.C. in April, 1960 and from Epping, Essex in April, 1968. The cones were allowed to dry for 1 or 2 days on sheets of filter paper, and were then shaken to remove the spores.

Isolation of Equisetolic Acid

Air-dried spores (20·83 g) of *E. arvense** were gently shaken with CH_2Cl_2 (225 ml) for 10 min at room temp., and the mixture was then filtered through a thin layer of Celite. On evaporation the filtrate gave 67 mg of a wax which was shown by TLC to be a mixture of free acids, polar substances, wax esters, and hydrocarbons and which is being examined further. The spores were continuously extracted (Soxhlet) with Et_2O for 7 days. The solution (1400 ml) was concentrated to a small volume (*ca.* 60 ml) and the white solid (56 mg, m.p. 126–127°) which separated was filtered off. The filtrate was taken to dryness, and the residue recrystallized from dioxan to constant melting point to give a further quantity (28 mg, m.p. 126–127°) of *equisetolic acid* (lit.[14] m.p. for triacontanedioic acid = 125–126°). (Found: C, 74·1; H, 11·9; O, 13·5. $C_{30}H_{58}O_4$ requires C, 74·6; H, 12·1; O, 13·3%.) ν 3500–2400 (broad, OH stretching), 1703 (C=O stretching), 1300–1180 (fatty acid progression), 944, 730, 720 cm^{-1}.

The Na salt of equisetolic acid precipitated when the hot EtOH solution of the acid was basified with aqueous alcoholic NaOH. It was washed with H_2O and EtOH to give a colourless solid, decomposing at *ca.* 258°. ν 1556 cm^{-1}.

Dimethyl Equisetolate

Recrystallized equisetolic acid (50 mg) was dissolved in a mixture of MeOH (63 ml), benzene (31 ml) and acetone (31 ml). An excess of ethereal CH_2N_2 was added, and the solution was left overnight, then concentrated. The residue was crystallized from MeOH–CH_2Cl_2 to give colourless plates (32 mg) of *dimethyl equisetolate*, m.p. 83–84°. (Found: C, 75·1; H, 12·4; O, 12·7%, M^+ = 510·465. $C_{32}H_{62}O_4$ requires C, 75·2; H, 12·2; O, 12·5%, M = 510·465.) The mass spectrum (Fig. 2) was identical with that of synthetic dimethyl triacontanedioate. τ($CDCl_3$) 6·39 (s, $-COOMe$, 6H), 7·75 (unresolved, $-CH_2CO$, 4H), and 8·73 (bs, methylene chain). ν (Nujol) 1746 (C=O stretching), 1350–1180 (band progression), 730 and 720 cm^{-1}.

Examination of Homologues

Air dried spores (20 g) of *E. arvense* were washed with $CHCl_2$ as before, and then extracted continuously with Et_2O for 2 days. The Et_2O was conc. to about 250 ml, filtered, and evaporated to yield crude equisetolic acid (197 mg). (*E. telmateia* spores (10 g), similarly treated, gave 80 mg of crude equisetolic acid). The crude acid was dissolved in Et_2O (800 ml) and an excess of CH_2N_2 was added. Removal of the solvent, dissolution in CH_2Cl_2, filtration, and evaporation gave the crude dimethyl ester (190 mg), m.p. 80–84°.

* *E. telmateia* may be used, with similar results.

[12] G. Eglinton and R. J. Hamilton, *Science* **156**, 1322 (1967).

[13] J. C. Willis, *A Dictionary of the Flowering Plants and Ferns*, 7th ed, p. 413, Cambridge University Press, Cambridge (1966).

[14] K. Siegler and W. Hechelhammer, *Annalen* **528**, 114 (1937).

The crude dimethyl ester preparations were analysed by GLC using a column of 5% Apeizon L on 80–100 Gas-Chrom Z (60 × 0·6 cm, 240°, argon carrier gas at 20 psi) and a Pye-Argon instrument. The column was deactivated by injecting hexamethyldisilazane (2 × 0·05 ml) at 240° and then maintained at 240°/2 psi carrier gas pressure for 12 hr before use. The results of the analyses of the two crude esters are presented in Table 1. Synthetic dimethyl triacontanedioate did not separate from the major component of either crude ester sample on coinjection. The crude esters were also analysed by conversion to the corresponding hydrocarbon mixtures ($\xrightarrow{LiAlH_4}$ diol $\xrightarrow{TsCl}$ ditosylate $\xrightarrow{LiAlH_4}$ hydrocarbon) followed by GLC, with broadly similar results.

The two main components of the crude ester from *E. telmateia* were separated on a preparative scale on a column of 8% Apiezon L on 80–100 Gas-Chrom Z (90 × 0·6 cm, 250°, 120 ml/min). The C_{30}-diester was recrystallized from CH_2Cl_2–MeOH to give colourless plates, m.p. 84–85°. The C_{20}-diester was obtained in crystalline form, but still contaminated with about 10% of the C_{30}-diester. The mass spectrum showed a molecular ion at *m/e* 482·432 ($C_{30}H_{58}O_4$ requires 482·434) and the fragmentation pattern [*m/e* 451 (M-31), 450 (M-32), 418 (M-64), 376 (M-106), and a series of ions at 84 + 14n, 59 + 14n, 27 + 14n] corresponded to that expected for dimethyl octacosanedioate.

Anodic Synthesis of Dimethyl Thapsate, $CO_2Me(CH_2)_{14}CO_2Me$

Ethyl hydrogen azelate (20·75 g) in MeOH (20 ml) was treated with NaOMe (2·14 g sodium in 30 ml methanol) and H_2O (8 ml) added. The solution was electrolysed between a rotating circular Pt anode (16 cm^2) and a Hg cathode in a water-cooled cell for 2 hr at a current density of 0·125 amp/cm^2. The solution was poured into H_2O (500 ml) and extracted with Et_2O. The ethereal extract was washed thoroughly with $NaHCO_3$ soln., then with H_2O, and dried ($MgSO_4$). Evaporation of the solvent gave the crude diester (14·43 g) which was shown by GLC to contain a mixture of dimethyl thapsate, methyl ethyl thapsate, and diethyl thapsate.

The transesterification was completed by treating the crude ester (40·89 g) with conc. H_2SO_4 (5 ml) in MeOH (100 ml) under reflux for 15 hr. The MeOH was removed under reduced pressure, and the residue was poured into H_2O, extracted into Et_2O, and worked up as described above. The residue was distilled to give 17·8 g (42%) of dimethyl thapsate, b.p. 148–155°/0·15 mm, m.p. 49–50° (lit.[15] m.p. 51·6°).

Methyl Hydrogen Thapsate

A solution of dimethyl thapsate (17·7 g), MeOH (148 ml) and 1·01 *N*-methanolic $Ba(OH)_2$ (160 ml) was kept at 22° for 21 hr, when it had become practically neutral. The precipitated Ba salt was filtered off and washed with Et_2O. Treatment of the solid with 2 N HCl was followed by Et_2O extraction. The extract was washed with H_2O, dried and concentrated. The residue was crystallized from benzene to give methyl hydrogen thapsate (13·77 g, 81%), m.p. 70–71° (lit.[16] m.p. 65–67°).

Anodic Synthesis of Dimethyl Triacontanedioate (Dimethyl Equisetolate) (cf. *Refs.* 8, 15, 16).

A solution of methyl hydrogen thapsate (2·50 g) and K_2CO_3 (0·16 g) in MeOH (8 ml, warm to dissolve) was electrolysed between Pt electrodes (each of area 1·5 cm^2) in a cell equipped with a reflux condenser. The current was maintained at 0·3 amp for 2 hr. The mixture became hot, and frothed, and it was necessary to cool the cell by occasional cooling in an ice bath, care being taken not to precipitate the K salt. After removing MeOH, the product was suspended in H_2O, and extracted with CH_2Cl_2. The extract was washed with H_2O, dried (K_2CO_3) and concentrated, and submitted to short path distillation (b.p. 220–250°/0·05 mm). The distillate was crystallized from MeOH–CH_2Cl_2 to give colourless plates (766 mg, 36%) of dimethyl triacontanedioate, m.p. 84–85° (lit.[14] m.p. 82–83°). A small sample was subjected to GLC purification under the conditions described above. M ps 84–85°. Mixed m.p. with a similarly purified sample of dimethyl equiselolate = 84–85°. The IR spectra of the synthetic and natural diesters were identical.

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[15] P. CHUIT, *Helv Chim. Acta* **9**, 264 (1926).
[16] L. RUZICKA, M. STOLL and H. SCHINZ, *Helv. Chim. Acta* **11**, 1174 (1928).