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PLANT ANTICANCER AGENTS, XLIII. (*E,E*)-7,12-DIOXO-OCTADECA-8,10-DIEN-1-OIC ACID (OSTOPANIC ACID), A CYTOTOXIC FATTY ACID FROM *OSTODES PANICULATA*[1]

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In a previous report from these laboratories (2), we described the isolation and structure elucidation of two new cytotoxic tigliane esters from the stems and fruits of *Ostodes paniculata* Blume (Euphorbiaceae). At the same time, we also obtained a third cytotoxic constituent, but it was not until recently that we have examined this isolate in detail.

The molecular formula of this third compound, ostopanic acid, was established as $C_{18}H_{28}O_4$ through hrms, and the functionalities of the oxygen atoms were determined from the fully decoupled ^{13}C-nmr spectrum, which revealed two ketonic carbonyl groups (δ 200.9 and 200.8) and a carboxylic acid group (δ 176.0). The two remaining degrees of unsaturation in ostopanic acid were determined to be olefinic groups through the observation of two 2-carbon resonances at δ 135.8 and 138.9. A single methyl group was observed (δ 13.0). Ostopanic acid is, therefore, a linear fatty acid with two double bonds and two carbonyl groups. The location of these functionalities and the stereochemistry of the double bonds in ostopanic acid remained to be established.

The ^{1}H-nmr spectrum of ostopanic acid displayed a terminal methyl group at δ 0.93, and the homonuclear correlation (COSY) spectrum revealed coupling of this group with one of the four methylene resonances at δ 1.34. These latter signals displayed further coupling with an unresolved six-proton complex at δ 1.63. Three additional methylene groups were observed as triplets (J=7.3 Hz) at δ 2.35, 2.60, and 2.61, indicating that they were adjacent to carbonyl groups. In the COSY spectrum, each of these resonances was found to be coupled to the methylene resonances at ca. δ 1.6.

Four olefinic protons resonating at about δ 6.5 and 7.2 (2 H each) suggested the presence of two conjugated enone systems. Because these multiplets are not first order and do not display substantive coupling with the methylene protons, they may be interpreted as the AA′BB′ system of a conjugated diene-dione. At this point, therefore, the structure **2** could be proposed.

This partial structure assignment for ostopanic acid was supported by the ir and uv spectra. For example, the uv spectrum of **1** displayed a λ max 279 nm, corresponding well with that of octa-3,5-diene-2,7-dione. While the *Z,Z*- and *E,Z*-isomers absorb at longer wavelengths, the more stable *E,E*- form displays a λ max at 276 nm (3,4). In the ir spectrum, two bands attributable to an α,β-unsaturated ketone appeared at ν max 1680 and 1666 cm^{-1}. The observation of two bands is due to the conformational flexibility of the *E*- enone moiety (5). Strong absorption bands at 998 and 990 cm^{-1} indicated the *E,E*-relationship of the double bonds.

In the mass spectrum of ostopanic acid a molecular ion was observed at *m/z* 308. A McLafferty rearrangement in-

[1]For the previous paper in this series see Duh *et al.* (1).

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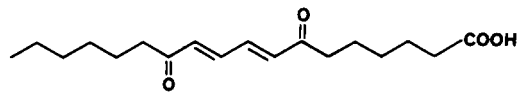

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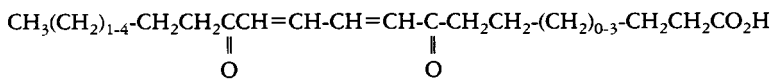

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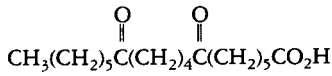

3

volving the carbonyl group at C-12 led to a fragment ion at *m/z* 238, and fragment ions at *m/z* 223 and 85 $(C_6H_{13})^+$ may be regarded as being due to the rupture of the C_{12}-C_{13} bond. The ions at *m/z* 238 and *m/z* 223 underwent a McLafferty rearrangement involving the carbonyl function at C-7, leading to the loss of $C_5H_8O_2$ and resulting in fragment ions at *m/z* 138 and *m/z* 123, respectively. Hrms indicated the ion at *m/z* 138 to have the molecular formula $C_8H_{10}O_2^+$. Loss of H_2O from the ions *m/z* 308 and *m/z* 223 led to ions at *m/z* 290 and 205, respectively. Rupture of the C_6-C_7 bond and subsequent elimination of CO from the resulting acylium ion led to an ion at *m/z* 165. Typical aliphatic ions were observed at *m/z* 57, 43, and 41. This evidence leads to structure **1** for ostopanic acid, i.e., (*E,E*)-7,12-dioxo-octadeca-8,10-dien-1-oic acid. Thus far we have been unable to assign the ^{13}C spectrum unambiguously, although preliminary attributions have been made by comparison with reference data for fatty acids (6,7).

The structure of ostopanic acid [**1**] was further confirmed from the spectral data of methyl tetrahydro-ostopanate (methyl 7,12-dioxo-octadecanoate) [**3**]. In comparison with **1**, the ^1H-nmr spectrum of **3** displayed additional signals at δ 2.39 and δ 1.54 (4 H each), attributable to the methylene groups at C-8, C-11, and C-9, C-10, respectively. In the ei mass spectrum obtained at 20 eV, a weak molecular ion was observed for **3** at *m/z* 326. Cleavage of the C_7-C_8 bond and subsequent elimination of OCH_3 led to the most intense fragment ion at *m/z* 126, and the base peak at *m/z* 85 was regarded as due to the rupture of the C_{12}-C_{13} bond. Two McLafferty rearrangements, involving the two carbonyl groups at C-7 and C-10, generated the characteristic ion at *m/z* 142.

Ostopanic acid [**1**] exhibited an ED_{50} of 1.5 μg/ml in the P-388 lymphocytic leukemia test system in vitro (8).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were determined using a Kofler hotstage microscope and are uncorrected. Uv spectra were recorded on a Beckman model DB-G spectrophotometer and ir spectra on a Nicolet model MX-1 FT-IR interferometer. ^1H-nmr spectra were recorded on a Nicolet NT-360 instrument at University of Illinois at Chicago. Mass spectra were obtained on a MAT 112S double focusing instrument (ei) or a Finnegan 4500 quadrupole instrument (ci).

PLANT MATERIAL.—Stems and fruits of *O. paniculata* were collected in India in December 1978, by staff members of the Economic Botany Laboratory, Agricultural Research Service, BARC-East, USDA, Beltsville, Maryland. A voucher specimen representing the collection has been deposited in the Herbarium of the National Arboretum, Washington, DC.

EXTRACTION AND FRACTIONATION.—The extraction and preliminary fractionation of *O. paniculata* have been described previously (2).

ISOLATION OF OSTOPANIC ACID [**1**].—The active fraction F-059 eluted with $CHCl_3$-MeOH

(49:1) from the chromatography of the $CHCl_3$ extract yielded ostopanic acid [**1**] on standing. An additional quantity of this compound was obtained through the further chromatographic purification of F-059 for a total yield of 150 mg (0.009%), mp 122-123°; ir ν max (KBr) 2950-2850, 1680, 1666, 1577, 1454, 1388, 1354, 1292, 1217, 1200, 998, 990, 720 cm^{-1}; uv λ max (log ϵ) 279 nm (4.52); ^1H nmr δ (360 MHz, $CDCl_3$) 0.93 (3H, t, J=7.3 Hz, 18-H_3) 1.34 (8H, m, 4, 15, 16, 17-H_2), 1.63 (6H, m, 3,5, 14-H_2), 2.35 (2H, t, J=7.2 Hz, 2-H_2), 2.60 (2H, t, J=7.2 Hz, 6-H_2 or 13-H_2), 2.61 (2H, t, J=7.3 Hz, 13-H_2 or 6-H_2), 6.47 (1H, m, 8-H or 11-H), 6.51 (1H, m, 11-H or 8-H), 7.16 (1H, m, 9-H or 10-H), 7.19 (1H, m, 10-H or 9-H); ^{13}C nmr δ (90.8 MHz, $CDCl_3$+CD_3OD) 200.9 (C-7 or C-12), 200.8 (C-12 or C-7), 176.0 (C-1), 138.9 (C-9 and C-10), 135.8 (C-8 and C-11), 40.7 (C-6 or C-13), 40.5 (C-13 or C-6), 33.8 (C-2), 29.3 (C-4, C-15 or C-16), 28.7 (C-15, C-16, or C-4), 28.6 (C-16, C-4 or C-15), 26.1 (C-3), 24.6 (C-5 or C-14), 23.8 (C-14 or C-5), 21.9 (C-17), 13.0 (C-18); ms *m/z* (rel. int.) ei 308 (M^+, 13), 290 (3), 223 (31), 207 (5), 205 (11), 165 (27), 138 (84), 137 (63), 125 (22), 123 (53), 95 (73), 85 (94), 81 (92), 67 (38), 57 (81), 55 (90), 53 (43), 52 (36), 43 (43), 41 (100); ci (CH_4) *m/z* 337 [$(M^+ + C_2H_5)^+$], 309 [$(M+H)^+$], and 291 [$(M+H)-18^+$]. Mass measurement: found 308.1983, calcd. for $C_{18}H_{28}O_4$ 308.1986; found 138.0681, calcd. for $C_8H_{10}O_2$ 138.0680.

SYNTHESIS OF METHYL 7,12-DIOXO-OCTADECANOATE [**3**].—A sample of ostopanic acid (**1**, 3 mg) in EtOH was subjected to catalytic hydrogenation (H_2, 5% Pd/C) followed by methylation of the residue with CH_2N_2. The methyl ester was purified on Si gel using $CHCl_3$ as eluent to afford **3**; ^1H nmr δ (360 MHz, $CDCl_3$) 0.90 (3H, t, J=7.3 Hz, 18-H_3), 1.30 (8H, m, 4, 15, 16, 17-H_2), 1.54 (10H, m, 3, 5, 9, 10, 14-H_2), 2.30 (2H, t, J=7.5 Hz, 2-H_2), 2.39 (8H, m, 6,8,11,13-H_2), 3.66 (3H, s, 1-CO_2CH_3); ms *m/z* (rel. int.) 326 (M^+, 3), 227 (25), 226 (22), 195 (35), 185 (31), 184 (27), 169 (1), 142 (31), 126 (73), 125 (27), 123 (42), 111 (14), 97 (25), 85 (79), 84 (100), 57 (43), 55 (25), 43 (15).

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LITERATURE CITED

1. C.Y. Duh, C.H. Phoebe, Jr., J.M. Pezzuto, A.D. Kinghorn, and N.R. Farnsworth, *J. Nat. Prod.*, **49**, in press.
2. S.S. Handa, A.D. Kinghorn, G.A. Cordell, and N.R. Farnsworth, *J. Nat. Prod.*, **46**, 123 (1983).
3. P. Karrer, Ch. Eugster, and S. Perl, *Helv. Chim. Acta.*, **32**, 1013 (1949).
4. S.T. Davies and G.H. Witham, *J. Chem. Soc., Perkin Trans. I*, 1346 (1977).
5. A.F. Kluge and P. Lillya, *J. Org. Chem.*, **36**, 1977 (1971).
6. E. Wenkert, B.L. Buckwalter, J.R. Burkitt, M.J. Gašić, H.E. Gottlieb, E.W. Hagaman, F.M. Schell, and P.M. Wovkulich, in: "Topics in ^{13}C-NMR Spectroscopy," vol. 2. Ed. by G.C. Levy, J. Wiley and Sons, New York, 1976, p. 81.
7. L. Bergter and P.R. Seidl, *Lipids*, **19**, 44 (1984).
8. R.I. Geran, N.H. Greenberg, M.M. MacDonald, A.M. Schumacher, and B.J. Abbott, *Cancer Chemother. Rep.*, **3** (2), 1 (1972).

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