Extractives of Fungi. Part III.[1] Introduction of a (*Z*)-17(20)-Double Bond into a Tumulosic Acid Derivative

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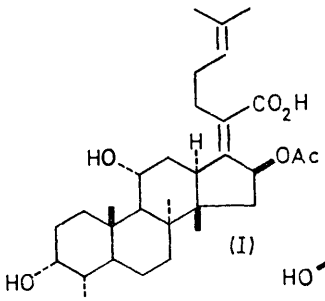
A method is described for converting tumulosic acid into (17*Z*)-3-oxoeburica-8,17(20)-dien-21,16α-olactone (XVI).

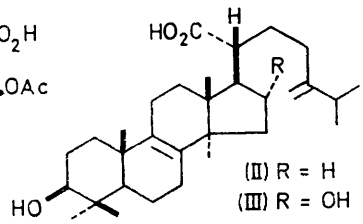
We have been investigating methods for introducing some of the structural features of the triterpenoid antibiotic, fusidic acid (I)[2-4] into readily available fungal acids such as eburicoic acid (II) and tumulosic acid (III). Tumulosic acid was of particular interest in view of our finding[5] that the basidiomycete fungus, *Trametes lilacino gilva*, is a prolific source of this compound, and because of its close relationship to polyporenic acid (IV), which has been shown to have weak antibiotic activity.[6] In model experiments connected with this work we have found two methods[7,8] for removing a 4-methyl group from lanosterol derivatives, and a route from a 4-demethyl-lanostane derivative to a compound with the fusidane skeleton.[9] We now report the introduction into a tumulosic acid derivative of a (*Z*)-17(20)-double bond, a feature of fusidic acid which structure–activity studies have shown[4] to be necessary for high activity.

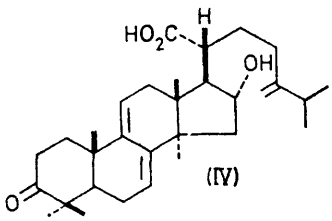
An obvious approach was dehydrobromination of a suitable 17- or 20-bromo-derivative. The starting material initially chosen was methyl 3β-acetoxy-16-oxoeburic-8-en-21-oate (V).[5] However, attempts to brominate this ketone or to prepare the enol acetate were unsuccessful. This is not altogether surprising in view of the difficulty in obtaining enol acetates of steroidal 16-ketones.[10] Introduction of a 17(20)-double bond into the keto-ester (V) with selenium dioxide, chloranil, or DDQ under a variety of conditions was also unsuccessful. In another approach methyl 3β-acetoxy-16α-hydroxyeburic-8-en-21-oate (VI)[5] was reduced with lithium aluminium hydride to the triol (VII), which on careful oxidation with Jones reagent yielded the diketo-aldehyde (VIII). However, this compound (VIII) and its 3-ethylene acetal were also resistant to dehydrogenation with selenium dioxide and DDQ. The configuration at C(20) in keto-al (VIII) was as indicated since methylation of the acid (IX), obtained on further oxidation of (VIII), gave a diketo-ester (X) identical

[1] Part II, J. T. Pinhey, B. J. Ralph, J. J. Simes, and M. Wootton, *Austral. J. Chem.*, 1971, **24**, 609.
[2] W. O. Godtfredsen and S. Vangedal, *Tetrahedron*, 1962, **18**, 1029.
[3] W. O. Godtfredsen, W. von Daehne, S. Vangedal, A. Marquet, D. Arigoni, and A. Melera, *Tetrahedron*, 1965, **21**, 3505.
[4] W. O. Godtfredsen, W. von Daehne, L. Tybring, and S. Vangedal, *J. Medicin. Chem.*, 1966, **9**, 15.
[5] J. T. Pinhey, B. J. Ralph, J. J. H. Simes, and M. Wootton, *Austral. J. Chem.*, 1970, **23**, 2141.
[6] P. Boiteau, B. Pasich, and A. R. Ratsimamanga, 'Les Triterpenoids,' Gauthier–Villars, Paris, 1964, pp. 106—1071.
[7] R. Kazlauskas, J. T. Pinhey, J. J. H. Simes, and T. G. Watson, *Chem. Comm.*, 1969, 945.
[8] K. F. Cohen, R. Kazlauskas, and J. T. Pinhey, *Chem. Comm.*, 1971, 1419.
[9] R. Kazlauskas, J. T. Pinhey, and J. J. H. Simes, *J.C.S. Perkin I*, 1972, 1243.
[10] J. Fishman, *J. Amer. Chem. Soc.*, 1960, **82**, 6143.

with the Jones oxidation product of methyl 3β,16α-dihydroxyeburic-8-en-21-oate. Bromination of the 3-ethylene acetal of the aldehyde (VIII) was another route

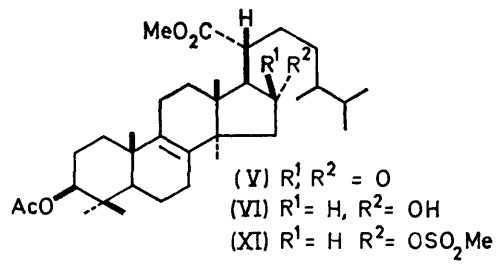


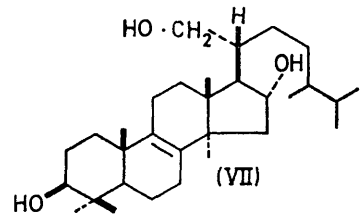


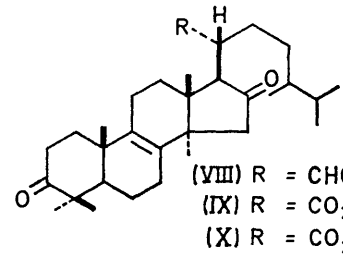


investigated, but here again a bromo-derivative could not be obtained.

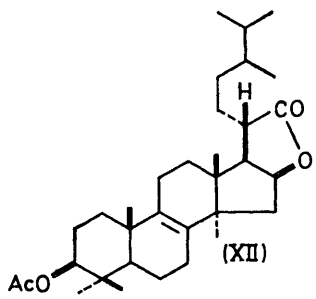
In a second approach to the introduction of a 17(20)-double bond, we envisaged the dehydration of 16-hydroxy-ester (VI) to a 16-olefin, followed by epoxidation and β-elimination to yield a compound with a 16-hydroxy-group and the required double bond. However, treatment of the mesylate derivative (XI) with 2,4,6-trimethylpyridine led to the formation of the lactone (XII), identical with that obtained by treatment of the hydroxy-ester (VI) with thionyl chloride, con-

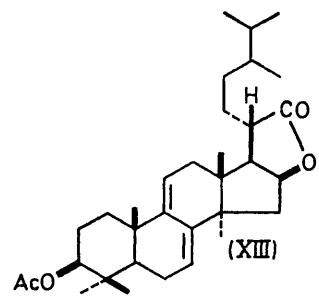


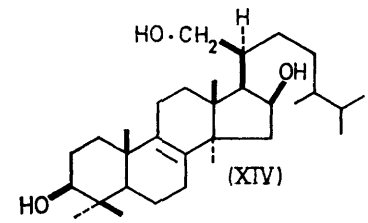


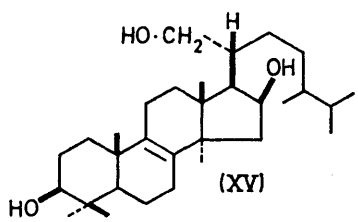


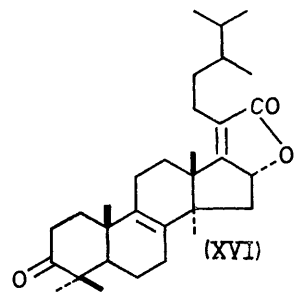
ditions previously used[11] for converting methyl 16α-hydroxy-3-oxoeburica-7,9(11)-dien-21-oate into the lactone (XIII). The stereochemistry at C(20) in the lactone (XII) was shown to be the same as that in

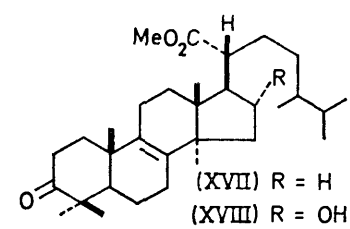












[11] A. Bowers, T. G. Halsall, and G. C. Sayer, *J. Chem. Soc.*, 1954, 3070.

(XIII) [11] (*i.e.* opposite to that in tumulosic acid) in the following way. Reduction with lithium aluminium hydride of the lactone (XII) gave a triol (XIV), which was different from either the 16α-triol (VII) or 16β-triol (XV); the latter was formed together with (VII) on reduction of the 16-ketone (V) with lithium aluminium hydride.

Introduction of the required 17(20)-double bond was eventually achieved by heating the diketo-acid (IX) under reflux for 8 days in benzene containing toluene-*p*-sulphonic acid. The only product isolated from this reaction was the αβ-unsaturated lactone (XVI) in 48% yield. The structure of (XVI) followed from the i.r. ($\nu_{max.}$ 1758 and 1703 cm^{-1}) and u.v. [$\lambda_{max.}$ 226 nm (ε 11,400)] spectra, while the proposed 16α-configuration rests on its hydrogenation over palladium catalyst to produce, after mild hydrolysis and methylation with diazomethane, methyl 3-oxoeburic-8-en-21-oate [12] (XVII) and the 16α-hydroxy-derivative (XVIII). The structure of the latter compound (XVIII) was determined by reduction with sodium borohydride to methyl 3β,16α-dihydroxyeburic-8-en-21-oate.[13]

The very slow formation of the αβ-unsaturated lactone (XVI) is probably due to the previously mentioned reluctance of 16-ketones to form derivatives of the enol. We did not detect (i.r.) the presence of the intermediate enol lactone under the conditions used.

EXPERIMENTAL

M.p.s were taken on a Kofler hot-stage apparatus. Unless otherwise stated, u.v. spectra were determined for solutions in ethanol, i.r. spectra were obtained for Nujol mulls, and rotations were measured for 1% solutions in chloroform. N.m.r. spectra were recorded on either Varian A60 or HA100 instruments with *ca.* 10% solutions in deuteriochloroform, and data are given in the following manner: chemical shift from internal reference (tetramethylsilane), multiplicity (exch. means exchanges on shaking with D_2O). Mass spectra were obtained with an A.E.I. MS902 instrument operated at 70 eV. Silica gel used for column chromatography was Davisons grade 923, while alumina refers to Spence type H. Light petroleum refers to the fraction of b.p. 60—80°. Analyses were carried out by Dr. E. Challen, University of New South Wales, and the Australian Microanalytical Service, Melbourne.

Reduction with Lithium Aluminium Hydride of Methyl 3β-Acetoxy-16α-hydroxyeburic-8-en-21-oate (VI).—The methyl ester (VI) (0·5 g) in ether (100 ml) was stirred at room temperature with lithium aluminium hydride (0·3 g). Ethyl acetate was added to decompose the excess of reagent, and the reaction was worked-up in the usual way to yield *eburic-8-ene-3β,16α,21-triol* (VII) (0·35 g), m.p. 238—240° (from methanol), $[\alpha]_D$ (pyridine) + 40°, $\nu_{max.}$ 3400 cm^{-1} (Found: C, 78·3; H, 11·2. $C_{31}H_{54}O_3$ requires C, 78·4; H, 11·5%).

3,16-Dioxoeburic-8-en-21-al (VIII).—Eburic-8-ene-3β,16α,21-triol (1·0 g) in acetone (100 ml) was treated with Jones reagent at room temperature until an orange colour persisted. The usual work-up gave *3,16-dioxoeburic-8-en-21-al* (0·55 g), m.p. 156—158° (from methanol), $[\alpha]_D$ +15°, $\nu_{max.}$ 1740 and 1715 cm^{-1}, $\nu_{max.}$ ($CHCl_3$) 1735, 1723, and 1703 cm^{-1}, δ 9·5 p.p.m. (1H, d, *J* 4 Hz, CHO) (Found: C, 78·2; H, 10·2. $C_{31}H_{48}O_3,0{\cdot}5H_2O$ requires C, 78·0; H, 10·3%).

3,3-Ethylenedioxy-16-oxoeburic-8-en-21-al.—Methyl 3,16-dioxoeburic-8-en-21-oate (0·4 g) in benzene (40 ml) was heated under reflux (Dean and Stark trap) for 6 h with ethylene glycol (5 ml) and toluene-*p*-sulphonic acid (60 mg). The cooled solution was washed with dilute sodium hydrogen carbonate solution, and the product was purified by chromatography on silica gel. *Methyl 3,3-ethylenedioxy-16-oxoeburic-8-en-21-oate* (230 mg) was obtained as a gum, $[\alpha]_D$ −31°, $\nu_{max.}$ ($CHCl_3$) 1732 cm^{-1}, δ 3·62 (3H, s, CO_2Me) and 3·40br p.p.m. (4H, s, ·O·CH_2·CH_2·O·).

Methyl 3,3-ethylenedioxy-16-oxoeburic-8-en-21-oate (200 mg) in anhydrous ether (200 ml) was heated under reflux for 4 h with lithium aluminium hydride (200 mg). The usual work-up gave *16ξ,21-dihydroxyeburic-8-en-3-one 3-ethylene acetal* (110 mg), m.p. 110—113° (needles from acetone–light petroleum), $[\alpha]_D$ +16°, $\nu_{max.}$ ($CHCl_3$) 3480 and 3400 cm^{-1}, δ 3·3 (6H, m, −CH_2·OH and ·O·CH_2·CH_2·O·) and 4·3 p.p.m. (1H, m, C*H*·OH) (Found: C, 76·9; H, 10·9%; *M*, 516·7806. $C_{33}H_{56}O_4$ requires C, 76·7; H, 10·9%; *M*, 516·7798).

16ξ,21-Dihydroxyeburic-8-en-3-one 3-ethylene acetal (100 mg) in pyridine (5 ml) was added with stirring to chromium trioxide (28 mg) in pyridine (1 ml). The mixture was stirred at room temperature for 5 h, diluted with water, and extracted with ether. After washing with cold dilute hydrochloric acid and saturated sodium hydrogen carbonate solution, the extract afforded 3,3-ethylenedioxy-16-oxoeburic-8-en-21-al (52 mg) as a gum, $[\alpha]_D$ ±0°, $\nu_{max.}$ ($CHCl_3$) 1736 and 1719 cm^{-1}, δ 3·4br (4H, s, ·O·CH_2·CH_2·O·) and 9·2 p.p.m. (1H, d, *J* 4 Hz, CHO).

3,3-Ethylenedioxy-16-oxoeburic-8-en-21-al (20 mg) in methanol (2 ml) containing dilute sulphuric acid (3 drops) was heated on the steam-bath for 10 min. Dilution with water and extraction with ether yielded 3,16-dioxoeburic-8-en-21-al, m.p. and mixed m.p. 156—157°, i.r. spectrum identical with that of material described above.

3,16-Dioxoeburic-8-en-21-oic Acid (IX) *and its Methyl Ester* (X).—The aldehyde (VIII) (400 mg) in acetone (100 ml) was treated with Jones reagent on the steam-bath until an orange colour persisted. The usual work-up afforded *3,16-dioxoeburic-8-en-21-oic acid* (0·29 g), m.p. 229—231° (from aqueous methanol), $[\alpha]_D$ −60°, $\nu_{max.}$ 3000, 1745, 1710, and 1660 cm^{-1}, δ 10·3br p.p.m. [1H, (exch.), CO_2H] (Found: C, 76·8; H, 9·7. $C_{31}H_{48}O_4$ requires C, 77·0; H, 9·9%).

Treatment of the foregoing acid (IX) (200 mg) with an ether solution of diazomethane yielded the *methyl ester* (150 mg), m.p. 169—171° (plates from aqueous methanol), $[\alpha]_D$ −10°, $\nu_{max.}$ 1735 and 1710 cm^{-1}, δ 3·7 p.p.m. (3H, s, CO_2Me) (Found: C, 75·35; H, 9·7%; *M*, 498·366. $C_{32}H_{50}O_4,0{\cdot}5H_2O$ requires C, 75·7; H, 10·1%; *M*, for $C_{32}H_{50}O_4$, 498·371).

Methyl 3β,16α-dihydroxyeburic-8-en-21-oate (500 mg) in acetone (50 ml) was treated at room temperature with Jones reagent until an orange colour persisted. The usual work-up afforded methyl 3,16-dioxoeburic-8-en-21-oate (450 mg), m.p. 169—171°, undepressed on admixture with material obtained above (i.r. spectrum identical).

[12] J. S. E. Holker, A. D. G. Powell, A. Robertson, J. J. H. Simes, and J. S. Wright, *J. Chem. Soc.*, 1953, 2414.

[13] J. M. Guider, T. G. Halsall, R. Hodges, and E. R. H. Jones, *J. Chem. Soc.*, 1954, 3234.

3β-*Acetoxyeburic*-8-*en*-21,16β-*olactone* (XII).—Methyl 3β-acetoxy-16α-hydroxyeburic-8-en-21-oate (VI) (0·9 g) in dry pyridine (10 ml) and chloroform (10 ml) was stirred at 0° with methanesulphonyl chloride (1 ml) for 4 h, and the mixture was then poured onto ice. The product was extracted into ether, the extract was washed with dilute hydrochloric acid, saturated sodium hydrogen carbonate solution, and water, and dried. The non-crystalline material obtained on removal of the solvent was used in the next step without further purification.

The crude mesylate obtained above (0·88 g) was heated under reflux for 6 h in toluene (20 ml) containing 2,4,6-trimethylpyridine (18 ml). The solution was cooled, diluted with ether (50 ml), and washed with dilute hydrochloric acid, saturated sodium hydrogen carbonate solution, and water. The product in light petroleum was adsorbed on a column of alumina (30 g), and elution with benzene–light petroleum (1 : 1) gave 3β-*acetoxyeburic*-8-*en*-21,16β-*olactone* (0·55 g), m.p. 207—210° (from methanol), $[\alpha]_D$ +25°, $\nu_{max.}$ 1770, 1735, and 1245 cm^{-1}, δ 2·0 (3H, s, OAc), 2·7 (1H, m, 20-H), 4·5 (1H, dd, *J* 4 and 10 Hz, 3α-H), and 4·8 p.p.m. (1H, m, 16α-H) (Found: C, 77·0; H, 10·1. $C_{33}H_{52}O_4$ requires C, 77·3; H, 10·2%).

A solution of methyl 3β-acetoxy-16α-hydroxyeburic-8-en-21-oate (0·3 g) in benzene (30 ml) containing thionyl chloride (0·3 ml) was heated under reflux for 2 h. The reaction was worked-up as before and the product chromatographed in the same way to afford the lactone (XII) (80 mg), m.p. 207—210°, undepressed on admixture and i.r. spectrum identical.

Reduction with Lithium Aluminium Hydride of the Lactone (XII).—The lactone (XII) (100 mg) in dry ether (100 ml) was heated under reflux with lithium aluminium hydride (80 mg) for 2 h, and the reaction worked-up in the usual way to give 20-*epieburic*-8-*ene*-3β,16β,21-*triol* (XIV) (55 mg), m.p. 237—239° (from methanol), $[\alpha]_D$ (pyridine) + 34°, $\nu_{max.}$ ($CHCl_3$) 3380 cm^{-1}, δ 1·85br [3H, (exch.), 3 × OH], 3·3 (3H, m, 3α-H, CH_2·OH), and 4·1 p.p.m. (1H, m, 16α-H), *m/e* 474 (22%), 459 (18), 441 (100), and 427 (59) (Found: C, 78·4; H, 11·8. $C_{31}H_{54}O_3$ requires C, 78·4; H, 11·5%).

Reduction with Lithium Aluminium Hydride of Methyl 3β-*Acetoxy*-16-*oxoeburic*-8-*en*-21-*oate*.—The 16-ketone (V) (0·3 g) in dry ether (100 ml) was stirred overnight at room temperature with lithium aluminium hydride (0·2 g). The reaction was worked-up in the usual way and the product in benzene–chloroform (1 : 1) adsorbed on a column of alumina (35 g). Elution with benzene–chloroform (1 : 1) gave *eburic*-8-*ene*-3β,16β,21-*triol* (XV) (90 mg), m.p. 238—240° (from methanol), $[\alpha]_D$ (pyridine) +89°, $\nu_{max.}$ 3400 cm^{-1} (Found: C, 78·6; H, 11·3. $C_{31}H_{54}O_3$ requires C, 78·4; H, 11·5%). Further elution with benzene–chloroform (1 : 1) yielded eburic-8-ene-3β,16α,21-triol (VII) (100 mg), m.p. 236—237°, undepressed on admixture with the material obtained by reduction of the ester (VI), $[\alpha]_D$ (pyridine) +40° (i.r. spectrum identical).

(17Z)-3-*Oxoeburic*-8,17(20)-*dien*-21,16α-*olactone* (XVI).—3,16-Dioxoeburic-8-en-21-oic acid (150 mg) was heated under reflux for 8 days in benzene (100 ml) containing toluene-*p*-sulphonic acid (50 mg). The boiling solvent was allowed to percolate through molecular sieves (Linde type 4A, 8—12 mesh beads), which were replaced every 48 h. The solution was cooled, filtered through a column of alumina (3 g), and the solvent was removed. The residue was fractionated by preparative t.l.c. on silica gel to give (17Z)-3-*oxoeburic*-8,17(20)-*dien*-21,16α-*olactone* (70 mg, 48%), m.p. 224—226° (from hexane), $[\alpha]_D$ −12°, $\lambda_{max.}$ 226 nm (ε 11,400), $\nu_{max.}$ ($CHCl_3$) 1758 and 1703 cm^{-1}, δ 5·1 p.p.m. (1H, m, 16β-H), *m/e* 466 (63%), 451 (100), and 435 (71) (Found: C, 79·8; H, 9·7. $C_{31}H_{46}O_3$ requires C, 79·8; H, 9·9%).

Hydrogenation of the Lactone (XVI).—The lactone (XVI) (93 mg) in ethyl acetate (15 ml) was hydrogenated over 10% palladium–charcoal (8 mg) until the uptake of hydrogen had ceased. The residue obtained after removal of the catalyst and solvent was warmed for 10 min on the steam-bath with 5% methanolic potassium hydroxide solution (5 ml). The solution was then acidified with dilute hydrochloric acid and the product, which was isolated by extraction with ether, was methylated by treatment with ethereal diazomethane. Separation of the methylated material by preparative t.l.c. on silica gel afforded two fractions. The less polar material crystallised from aqueous methanol to give methyl 3-oxoeburic-8-en-21-oate (18 mg), m.p. 121—123°, undepressed on admixture with an authentic sample prepared from methyl 3β-hydroxyeburic-8-en-21-oate, $[\alpha]_D$ +56° (lit.,[12] m.p. 123—124°, $[\alpha]_D$ +61°). The more polar fraction crystallised from methanol to give *methyl* 16α-*hydroxy*-3-*oxoeburic*-8-*en*-21-*oate* (XVIII) (25 mg), m.p. 192—193° (needles), $[\alpha]_D$ +14°, $\nu_{max.}$ ($CHCl_3$) 1726 and 1701 cm^{-1}, δ 3·67 (3H, s, CO_2Me) and 4·1 p.p.m. (1H, m, 16β-H) (Found: C, 76·6; H, 10·4. $C_{32}H_{52}O_4$ requires C, 76·75; H, 10·5%).

Reduction with Sodium Borohydride of Methyl 16α-*Hydroxy*-3-*oxoeburic*-8-*en*-21-*oate* (XVIII).—Sodium borohydride (15 mg) was added to a solution of the ketone (XVIII) (20 mg) in methanol (3 ml) and dioxan (3 ml) and the mixture stirred at room temperature for 2 h. The material precipitated on dilution with water crystallised from methanol to give methyl 3β,16α-dihydroxyeburic-8-en-21-oate (methyl dihydrotumulosate), m.p. 181—182°, undepressed on admixture with an authentic sample, $[\alpha]_D$ +25° (lit.,[13] m.p. 173—174°, $[\alpha]_D$ +25°) (i.r. spectrum identical).

This work was supported by a grant from the Australian Research Grants Committee.

[2/704 *Received*, 27*th March*, 1972]