Acerotin and Acerocin, Novel Triterpene Ester Aglycones from the Tumour-inhibitory Saponins of *Acer negundo*[1]

By S. MORRIS KUPCHAN,* MITSUO TAKASUGI, ROGER M. SMITH, and P. S. STEYN

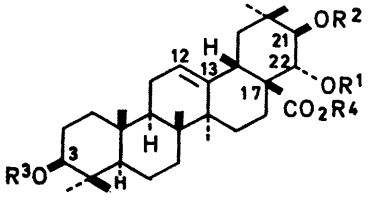
(*Department of Chemistry, University of Virginia, Charlottesville, Virginia* 22901, *and Department of Pharmaceutical Chemistry, University of Wisconsin, Madison, Wisconsin* 53706)

Summary The two major aglycones (I) and (II) from the tumour inhibitory saponins of *Acer negundo* are shown to be diesters of a new triterpene; each aglycone yields acetic acid and a unique nonadienoic acid upon hydrolysis.

IN the course of a continuing search for tumour inhibitors of plant origin, systematic fractionation of an extract of *Acer negundo* L. yielded single-spot acidic saponin P.[2] The material showed significant inhibitory activity against the sarcoma 180 and the Walker intramuscular carcinosarcoma 256 tumour systems,[2] and further testing indicated that saponin P is the most promising of the known tumour-inhibitory saponins.[3,4] We report here the structural elucidation of two novel triterpene ester aglycones, acerotin (I) and acerocin (II), obtained upon hydrolysis of saponin P.

Acid hydrolysis of saponin P yielded glucose and arabinose (detected by g.l.c. of their trimethylsilyl ethers) and a mixture of acidic aglycones. The aglycones were separated by preparative t.l.c. on silica gel and then on alumina to yield the major components: acerotin (I) and acerocin (II). Acerotin† (I), $C_{41}H_{62}O_7$, showed m.p. 240—243°; λ_{max} (MeOH) 264 nm (ϵ 28,400); λ_{max} (KBr) 5·73, 5·76, 5·87, 6·11, 6·20 μm; mass spectrum M^+ 666·4496 (required 666·4496). Acerocin (II), $C_{41}H_{62}O_7$, showed m.p. 205—207°; λ_{max} 266 nm (ϵ 22,900); λ_{max} (KBr) 5·71, 5·77, 5·81, 6·12, 6·26 μm; mass spectrum M^+ 666·4513 (required 666·4496). Further treatment of the aglycones with acid failed to cause any interconversion, indicative that saponin P was a mixture.

On alkaline hydrolysis, both aglycones (I) and (II) yielded acerogenic acid (III), $C_{30}H_{48}O_5$: m.p. 308—310°; u.v. end absorption 210 nm (ϵ 4400); λ_{max} (KBr) 5·90 μm; mass spectrum M^+ 488. On treatment with diazomethane the acid (III) formed a methyl ester (IV), $C_{31}H_{50}O_5$: m.p. 236—238°; λ_{max} (KBr) 5·82 μm, which on treatment with acetic anhydride in pyridine yielded the triacetate (V), $C_{37}H_{56}O_8$: m.p. 212—213°; λ_{max} (KBr) 5·69, 5·77, 8·04 μm; mass spectrum *m/e* 568 (M^+ — AcOH). Thus the oxygen atoms in the acid (III) were present as a carboxyl group and as three hydroxyl groups indicated by the n.m.r. spectrum of the acetate (V) to be secondary (τ 4.81 and 5·04, AB quartet, J = 10 Hz and τ 5·51, dd, J = 6, 9Hz). The n.m.r. spectrum (C_5D_5N) of the methyl ester (IV) contained signals for seven quaternary *C*-methyl groups, one olefinic proton (τ 4·50, m), and three protons on carbon bearing hydroxyl (τ 5·6–6·3, m). The mass spectra of the acid (III) and the ester (IV) corresponded well with a β-amyrin skeleton containing a 12,13-double bond. The typical[5] retro-Diels–Alder fragmentation gave ions at *m/e* 280 [294 in (IV)] and *m/e* 207 (from rings D and E and from rings A and B, respectively). The former ion lost 18, 36, or 46 mass units [18, 36, or 60 in (IV)] confirming the presence of the carboxyl and two hydroxyl groups in the D,E-ring system. The remaining hydroxyl group was assigned to C-3 on biogenetic grounds. The carboxyl group could be assigned to C-17, as treatment with bromine in methanol[6] converted the acid (III) to a bromo-γ-lactone: λ_{max} (KBr) 5·66 μm. The n.m.r. spectrum of the acetate (V) showed that the two hydroxyl groups in the D,E-ring system of the acid (III) constituted a diequatorial diol. These assignments were confirmed by reduction of the methyl ester (IV) with lithium aluminium hydride to yield 16-deoxybarringtogenol C, whose structure has been derived from an *X*-ray crystallographic study.[7]



(I) R^1 = Ac, R^2 = CO·[CH = CH]$_2$Bu^s, R^3 = R^4 = H (t)

(II) R^1 = Ac, R^2 = CO·CH = CH·CH=CHBu^s, R^3=R^4=H (c, t)

(III) R^1 = R^2 = R^3 = R^4 = H

(IV) R^1 = R^2 = R^3 = H, R^4 = Me

(V) R^1 = R^2 = R^3 = Ac, R^4 = Me

(VI) R^1 = H, R^2 = CO·[CH = CH]$_2$Bu^s, R^3 = R^4 = H (t)

(VII) R^1 = H, R^2 = CO·CH = CH·CH = CH, R^3 = R^4 = H (c, t)

(VIII) R^1 = Ac, R^2 = CO·[CH = CH]$_2$Bu^s, R^3 = Ac, R^4 = H (t)

(IX) HO_2C·CH = CH·CH = CHBu^s (t, t)

(X) HO_2C·CH = CH·CH = CHBu^s (c, t)

The alkaline hydrolysis of acerotin (I) also yielded the *trans,trans*-diene-acid (IX), which was isolated by g.l.c. after conversion into the optically-active methyl ester: λ_{max} 260 nm; λ_{max} ($CDCl_3$) 5·87, 6·10, 6·19, 10·00 (*trans*-olefin) μm; mass spectrum *m/e* 168 (M^+), 111 ($M^+ - C_4H_9$). Acerocin (II) yielded instead the isomeric optically active 2-*cis*-4-*trans*-diene-acid (X), which was isolated as the methyl ester: λ_{max} 263 nm; λ_{max} ($CDCl_3$) 5·87, 6·13, 6·26, 10·10 (*trans*-olefin), 10·42 μm (*cis*-olefin). The n.m.r. spectra of both compounds showed the presence of the s-butyl group and the coupling constants and chemical shifts of the olefinic protons were directly comparable to those of methyl 2,4-*trans,trans*- and 2-*cis*-4-*trans*-sorbate, respectively.[8] The u.v. and i.r. spectra agreed well with model compounds[9] and the *m/e* 111 peak was characteristic of a methyl $\alpha\beta,\gamma\delta$-diene-ester.[10]

In the n.m.r. spectra of the aglycones (I) and (II) the AB quartet appeared at τ 4·65 and 4·98, indicative that the ester functions are at C-21 and C-22. Partial hydrolysis of the aglycones (I) and (II) gave the deacetyl derivatives

† All new crystalline compounds have been characterised by concordant elemental analyses.

(VI) and (VII), respectively. Oxidation in each case with the Jones reagent gave a diketo-acid, which was rapidly decarboxylated on warming. Thus the diene-ester function was assigned to C-21 and the acetyl group to C-22. Acetylation of the deacetyl compound (VI) gave 3-acetylacerotin (VIII), demonstrating that ester exchange had not occurred.

In view of the recent demonstration of the importance of $\alpha\beta$-unsaturated carbonyl functions for the tumour-inhibitory activity of other natural products,[11] the unsaturated ester function may play a significant role in the activity of these saponins.

We thank Professor Yosioka of Faculty of Pharmaceutical Sciences, Osaka University, Toyonaka, Osaka, Japan, for an authentic sample of 16-deoxybarringtogenol C, and the National Cancer Institute and the American Cancer Society for financial support.

(*Received, May 27th*, 1970; *Com.* 801.)

[1] For previous part in the series "Tumor Inhibitors" see: S. M. Kupchan, T. J. Giacobbe, I. S. Krull, A. M. Thomas, M. A. Eakin, and D. G. Fessler, *J. Org. Chem.*, in the press.
[2] S. M. Kupchan, R. J. Hemingway, J. R. Knox, S. J. Barboutis, D. Werner, and M. A. Barboutis, *J. Pharm. Sci.*, 1967, **56**, 603.
[3] J. M. Venditti and B. J. Abbott, *Lloydia*, 1967, **30**, 332.
[4] J. L. Hartwell and B. J. Abbott, *Adv. Pharm. Chemotherapy*, 1969, **7**, 117.
[5] H. Budzikiewicz, J. M. Wilson, and C. Djerassi, *J. Amer. Chem. Soc.*, 1963, **85**, 3688.
[6] D. H. R. Barton and P. de Mayo, *J. Chem. Soc.*, 1954, 887.
[7] I. Yosioka, K. Imai, and I. Kitagawa, *Tetrahedron Letters*, 1967, 2577; W. Hoppe, A. Gieren, N. Brodherr, R. Tschesche, and G. Wulff, *Angew. Chem. Internat. Edn.*, 1968, **7**, 547.
[8] J. A. Elvidge and P. D. Ralph, *J. Chem. Soc.* (*B*), 1966, 243.
[9] L. Crombie, *J. Chem. Soc.*, 1955, 1007.
[10] W. K. Rohwedder, A. F. Mabrouk, and E. Selke, *J. Phys. Chem.*, 1965, **69**, 1711.
[11] S. M. Kupchan, *Trans. New York Acad. Sci.*, 1970, **32**, 85; S. M. Kupchan, D. C. Fessler, M. A. Eakin, and T. J. Giacobbe, *Science*, 1970, **168**, 376.