Tumor Inhibitors. LXII.[1] The Structures of Acerotin and Acerocin, Novel Triterpene Ester Aglycones from the Tumor Inhibitory Saponins of *Acer negundo*[2]

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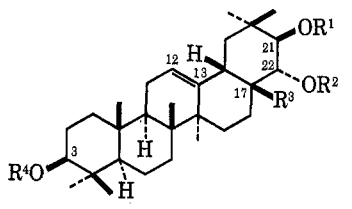
The aglycones acerotin (**1**) and acerocin (**2**) have been characterized as the major aglycones of the tumor inhibitory saponin P isolated from *Acer negundo* L. The two aglycones are diesters of the new acidic triterpene acerogenic acid (**3**). The structure of **3** was determined from its spectral properties and by chemical studies including conversion into 16-deoxybarringtogenol C. The ester groups were characterized as acetate and the novel nonadienoates corresponding to **11** and **12**, present in **1** and **2**, respectively. 24-Hydroxyacerogenic acid (**18**) has been characterized as one of the major sapogenins derived from the tumor inhibitory saponin Q. Hydrolysis of saponin Q also yielded acids **11** and **12**. The potential significance of the unsaturated ester functions for the growth inhibitory activity of the *Acer* saponins is discussed.

In the course of a continuing search for tumor inhibitors of plant origin, an ethanolic extract of the leaves and stems of *Acer negundo* L. (*Aceraceae*) has been shown to possess significant inhibitory activity. Systematic fractionation led to the isolation of the active principles as the chromatographically homogeneous acids, saponin P and saponin Q.[4] Saponin P was active against the sarcoma 180 and Walker intramuscular carcinosarcoma 256 tumor systems,[4] and on further testing the high therapeutic index in the latter system indicated "activity sufficient for recommendation as a clinical candidate."[5] The National Cancer Institute has since procreud a large collection of *Acer negundo* for extraction of *Acer* saponin P in a quantity sufficient for preclinical toxicological studies and preliminary clinical trials. The structural elucidation of the aglycones acerotin (**1**) and acerocin (**2**) formed by hydrolysis of saponin P have been briefly reported,[6] and we describe herein our detailed structural studies.

Acid hydrolysis of saponin P yielded glucose and arabinose, which were identified by vapor phase chromatography of their trimethylsilyl ethers, and a mixture of acidic aglycones. The aglycones showed only one major spot on examination by tlc on silica gel but on alumina showed two slightly separated major components. Fractionation first by tlc on silica gel and then repeatedly on alumina yielded the major components acerotin (**1**, R_f 0.57) and acerocin (**2**, R_f 0.55).

Elemental analysis and high-resolution mass spectrometry showed **1** and **2** to be isomeric $C_{41}H_{62}O_7$ compounds. The ultraviolet spectra (**1**, λ_{max} 264 mμ, and **2**, λ_{max} 266 mμ) were very similar, and the infrared spectra of both compounds contained bands assignable to a hydroxyl, a saturated ester, an unsaturated ester, a carboxyl, and a double bond (*i.e.*, **1**, 2.81, 2.94, 3.14, 5.73, 5.76, 5.87, 6.11, and 6.20 μ, respectively). The nmr spectra of **1** and **2** were very similar in the high field region and both contained an acetyl group signal at τ 8.06. Although both spectra contained an AB



R_{tt} = -CO(CH=CH)$_2$CH(CH$_3$)CH$_2$CH$_3$ (*t*)

R_{ct} = -CO(CH=CH)(CH=CH)CH(CH$_3$)CH$_2$CH$_3$ (*c*, *t*)

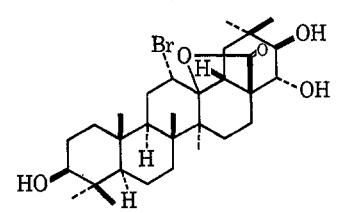
1, $R^1 = R_{tt}$; R^2 = Ac; $R^3 = CO_2H$; R^4 = H
2, $R^1 = R_{ct}$; R^2 = Ac; $R^3 = CO_2H$; R^4 = H
3, $R^1 = R^2 = R^4$ = H; $R^3 = CO_2H$
4, $R^1 = R^2 = R^4$ = Ac; $R^3 = CO_2H$
5, $R^1 = R^2 = R^4$ = H; $R^3 = CO_2CH_3$
6, $R^1 = R^2 = R^4$ = Ac; $R^3 = CO_2CH_3$
7, $R^1 = R^2 = R^4$ = H; $R^3 = CH_2OH$
8, $R^1 = R_{tt}$; $R^2 = R^4$ = H; $R^3 = CO_2H$
9, $R^1 = R_{ct}$; $R^2 = R^4$ = H; $R^3 = CO_2H$
10, $R^1 = R_{tt}$; $R^2 = R^4$ = Ac; $R^3 = CO_2H$

$CH_3O_2C(CH{=}CH)(CH{=}CH)CH(CH_3)CH_2CH_3$ (*t*, *t*)

11

$CH_3O_2C(CH{=}CH)(CH{=}CH)CH(CH_3)CH_2CH_3$ (*c*, *t*)

12



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quartet (τ 4.65 and 4.98, J = 10 Hz), there were marked differences in the τ 2–5 region. The mass spectra of the aglycones were virtually identical and both contained a base peak at m/e 137 and a strong peak at m/e 109, suggesting that a large ester grouping was being lost.

Alkaline hydrolysis of **1** and **2** yielded different unsaturated C_9 acids but the same acidic triterpene, acerogenic acid (**3**). Acerogenic acid, $C_{30}H_{48}O_5$, lacked the ultraviolet absorption and the mass spectral peaks at m/e 137 or 109 present in the spectra of the aglycones.

(1) Part LXI: T. Kurokawa, K. Nakanishi, W. Wu, H. Y. Hsu, M. Maruyama, and S. M. Kupchan, *Tetrahedron Lett.*, 2863 (1970).

(2) Supported by grants from the National Cancer Institute (CA-04500 and CA-11718) and the American Cancer Society (T-275).

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(4) S. M. Kupchan, R. J. Hemingway, J. R. Knox, S. J. Barboutis, D. Werner, and M. A. Barboutis, *J. Pharm. Sci.*, **56**, 603 (1967).

(5) J. M. Venditti and B. J. Abbott, *Lloydia*, **30**, 232 (1967).

(6) S. M. Kupchan, M. Takasugi, R. M. Smith, and P. Steyn, *Chem. Commun.*, 969 (1970).

The infrared spectra contained bands at 2.92 (OH) and 5.90 μ (carboxyl); the carboxylate salt showed bands at 6.38 and 7.20 μ. The nmr spectrum (pyridine-d_5) contained signals for three protons on carbon carrying hydroxyl (τ 5.6–6.3, m).

On acetylation **3** yielded a triacetate **4**, $C_{36}H_{54}O_8$, whose nmr spectrum contained signals assignable to acetyl groups at τ 7.96, 7.99, and 8.01 and to protons on carbon carrying acetoxyl at τ 4.70 and 5.06 (AB quartet, J = 11 Hz) and τ 5.51 (bt, J = 8 Hz). On treatment with diazomethane, **3** gave a methyl ester **5**, $C_{31}H_{50}O_5$, ir (KBr) 5.82 μ, whose nmr spectrum contained a signal at τ 6.25 (OMe). Acetylation of **5** yielded the triacetyl methyl ester **6**, $C_{37}H_{56}O_8$. The infrared spectrum of **6** lacked hydroxyl bands but contained bands at 5.69, 5.77 (ester), and 8.04 μ (acetate), and the nmr spectrum contained signals at τ 4.81 and 5.04 (AB quartet, J = 10 Hz) and τ 5.51 (dd, J = 6,9 Hz) corresponding to protons on carbon carrying acetoxyl, as well as signals for seven quaternary methyl groups, τ 8.84, 8.92, 9.07, 9.10, 9.13 (6 H), and 9.31, and one olefinic proton, τ 4.65 (bt, J = 7 Hz). Therefore, the oxygen atoms in **3** could be assigned to three secondary hydroxyl groups and a carboxyl group.

From the molecular formula and number of *C*-methyl groups present, **3** was proposed to have a β-amyrin skeleton containing a 12,13 double bond. The carboxyl group could be assigned to C-17, as on treatment of **3** with bromine in methanol[7] it formed the bromo-γ-lactone **13**, $C_{30}H_{47}BrO_5$. The infrared spectrum of **13** contained a band at 5.66 μ (γ-lactone) and the nmr spectrum lacked a signal assignable to an olefinic proton. The circular dichroism of acerogenic acid, λ 225 mμ ($\Delta\epsilon$ − 1.6), was found to be very similar to that reported for a series of Δ^{12}-triterpene-28-carboxylic acids,[8] indicative that it had a similar conformation.

These assignments were confirmed by the mass spectra of **3** and **5**, which exhibited a typical retro-Diels–Alder fragmentation of the C ring characteristic of the $\Delta^{12,13}$-β-amyrin skeleton.[9] Strong peaks were present at m/e 280, 262 (−18), 244 (−36), and 234 (−46) from **3**, and m/e 294, 276 (−18), 258 (−36), and 234 (−60) from **5**, demonstrating the presence of the carboxyl group and two hydroxyl groups in the D,E ring system. Significant peaks in both spectra at m/e 207 and 190 (−17) could be attributed to the A,B ring system carrying one hydroxyl group. On biogenetic grounds this hydroxyl was assigned to C-3 and the signal at τ 5.51 (dd, J = 6, 9 Hz) in the spectrum of **6** could be assigned to the C-3 proton. In the mass spectra of the triacetates **4** and **6** corresponding peaks appeared, with the appropriate fragmentations at m/e 364 and 378, respectively, for the D,E ring system and at m/e 190 (−60) for the A,B ring system.

From the nmr spectra the hydroxyl groups in the D,E ring system could be formulated as a diequatorial diol, as the coupling (J = 10 Hz) between the protons on carbon carrying oxygen suggested a diaxial relationship. If the D,E ring system is cis-fused and ring E is in a chair conformation, the diol could be located only at C-15,16 or C-21,22. C-15 α- and β-hydroxyl groups in derivatives of dumortierigenin[10] and the C-15 hydroxyl group in the 15α,16α-*cis*-diolentagenic acid[11] have been found to be acetylated only under conditions considerably more vigorous than those used to prepare **6**. Thus, the diol was tentatively assigned to C-21,22, and the novel structure **3** was proposed for the genin.

Confirmation of the oxygenation pattern and proposed stereochemistry was obtained by reduction of **5** with lithium aluminum hydride to yield the tetrol **7**, identical by direct comparison with 16-deoxybarringtogenol C.[12] The structure of 16-deoxybarringtogenol C has been determined by interrelation[13] with barringtogenol C, defined by X-ray crystallography of its diester.[14] Acetylation of **7** gave a tetraacetate, whose physical properties corresponded to those reported.[13]

The unsaturated acids formed on hydrolysis of the aglycones were first separated by tlc on silica gel and were then methylated by treatment with diazomethane. The methyl esters were purified by vapor phase chromatography and were collected as $CDCl_3$ solutions. Acerotin (**1**) yielded the optically active 2,4-*trans*-diene ester **11**. The ultraviolet spectrum, λ_{max} 260 mμ, and the infrared spectrum, 5.87, 6.10, 6.19, 10.00 μ (trans olefin), were very similar to those of methyl 2,4-*trans*-decanedienoate.[15] The nmr spectrum contained signals assignable to a *sec*-butyl group [τ 9.12 (t, J = 7 Hz, 3 H), 8.96 (d, J = 7 Hz, 3 H), 8.59 (quintet, J = 7 Hz, 2 H), and 7.81 (septet, J = 7 Hz, 1 H)], a methoxyl group [τ 6.28 (s, 3 H)], and four olefinic protons [τ 4.20 (d, J = 15.5 Hz, α H), 3.90–3.85 (m, γ and δ H), and 2.73 (dd, J = 15, 10 Hz, β H)]. The chemical shift and observable couplings of the olefinic protons agreed well with those reported for methyl 2,4-*trans*-sorbate [τ 4.32 (α H), 3.96 (γ H), 3.85 (δ H), 2.83 (β H), $J_{\alpha,\beta}$ = 15.8, $J_{\beta,\gamma}$ = 10.5 Hz][16] and differed from those reported for a 2-*trans*-4-*cis*-diene amide.[17] The mass spectrum contained a molecular ion (m/e 168) and strong peaks corresponding to loss of C_2H_5 (m/e 139) and C_4H_9 (m/e 111), whereas only small peaks were present for loss of CH_3 (m/e 153) and C_3H_7 (m/e 125), as expected for a compound containing a *sec*-butyl group.[18] The peak at m/e 111 is characteristic of an α,β : γ,δ-diene ester and can be represented as a pyrylium ion.[19]

Acerocin (**2**) yielded the isomeric optically active 2-*cis*-4-*trans*-diene ester **12**. The ultraviolet spectrum, λ_{max} 263 mμ, and infrared spectrum, λ_{max} 5.87, 6.13, 6.26, 10.10 (trans olefin), and 10.42 μ (cis olefin), were comparable to those of methyl 2-*cis*-4-*trans*-decadienoate.[15] The nmr spectrum contained signals for a *sec*-butyl group [τ 9.10 (t, J = 7 Hz, 3 H), 8.91 (d, J = 7 Hz, 3 H), 8.59 (quintet, J = 7 Hz, 2 H), 7.76 (septet, J = 7 Hz)], a methoxyl group [τ 6.26 (s, 3 H)], and four olefinic protons [τ 4.45 (d, J = 11 Hz, α H), 4.05

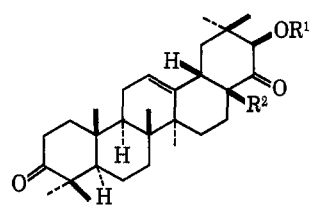
(7) D. H. R. Barton and P. de Mayo, *J. Chem. Soc.*, 887 (1954).
(8) J. D. Renwick, P. M. Scopes, and S. Huneck, *J. Chem. Soc. C*, 2544 (1969).
(9) H. Budzikiewicz, J. M. Wilson, and C. Djerassi, *J. Amer. Chem. Soc.*, **85**, 3688 (1963).
(10) C. Djerassi, C. H. Robinson, and D. B. Thomas, *ibid.*, **78**, 5685 (1956).
(11) A. K. Barua, P. Chakraborti, S. K. Pal, and B. C. Das, *J. Indian Chem. Soc.*, **47**, 195 (1970).
(12) We thank Professor Yosioka of the Faculty of Pharmaceutical Sciences, Osaka University, Toyonaka, Osaka, Japan, for an authentic sample of 16-deoxybarringtogenol C.
(13) I. Yosioka, K. Imai, and I. Kitagawa, *Tetrahedron Lett.*, 2577 (1967).
(14) W. Hoppe, A. Gieren, N. Brodherr, R. Tschesche, and G. Wulff, *Angew. Chem., Int. Ed. Engl.*, **7**, 547 (1968).
(15) L. Crombie, *J. Chem. Soc.*, 1007 (1955).
(16) J. A. Elvidge and P. D. Ralph, *ibid., B*,243 (1966).
(17) F. Bohlmann and M. Grenz, *Chem. Ber.*, **99**, 3197 (1966).
(18) R. Ryhage and E. Stenhagen, *Ark. Kemi*, **15**, 291 (1960).
(19) W. K. Rohwedder, A. F. Mabrouk, and E. Selke, *J. Phys. Chem.*, **69**, 1711 (1965).

(dd, J = 8, 16 Hz, δ H), 3.45 (t, J = 11.5 Hz, β H), 2.65 (dd, J = 11.5, 16 Hz, γ H)]. The olefinic couplings were confirmed by double irradiation and the chemical shifts and the couplings of the olefinic protons were comparable to the signals from the methyl 2-*cis*-4-*trans*-sorbate[16] [τ 4.52 (α H), 4.01 (δ H), 3.53 (β H), and 2.61 (γ H), $J_{\alpha,\beta}$ = 11.6, $J_{\beta,\gamma}$ = 11.5, $J_{\gamma,\delta}$ = 15.7, and $J_{\delta,\epsilon}$ = 7.05 Hz].

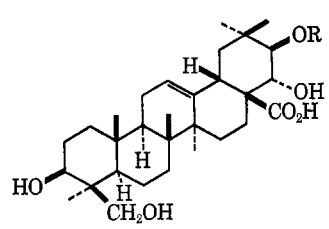
The differences in the olefinic proton signals of the two ester functions were clearly responsible for the differences between the nmr spectra of **1** and **2** and the presence of the corresponding olefin proton signals in the spectra of the aglycones confirmed that no isomerization of the esters had occurred on hydrolysis.[15] The formation of the same fragmentation ions in the mass spectra of the isomeric aglycones showed that the two ester functions also are isomeric.

As in the nmr spectra of the aglycones the AB quartet appeared at low field; the ester groupings were assigned to C-21 and C-22. Consequently, in saponin P the sugar moiety is located at C-3. In order to determine the relative orientation of the ester functions, partial acid hydrolysis of **1** and **2** was carried out to give the respective deacetyl derivative **8** and **9**. With alkali, only **3** could be isolated in good yield, as the intermediates were themselves rapidly hydrolyzed. The ultraviolet spectrum of **8** showed that it still contained the diene ester group but its nmr spectrum lacked signals for an acetyl group and the C-21,22 protons now appeared at τ 5.13 and 6.12 (AB quartet, J = 11 Hz). Acetylation of **8** yielded acetylacerotin (**10**), demonstrating that ester exchange had not occurred between C-21 and C-22. On tlc plates **9** absorbed ultraviolet light and thus also contained an unsaturated chromophore.

Jones oxidation of **8** and **9** afforded the corresponding diketo acids **14** and **15**, which on heating briefly were decarboxylated to yield the diketones **16** and **17**, respectively. The diketones were characterized by their mass spectra, which contained strong ions at m/e 137 and 109. Their ultraviolet spectra confirmed that they still contained the diene chromophore. Thus the acetoxyl group was located at C-22, β to the carboxyl group, and the diene ester could be assigned to C-21, giving the complete structure of the aglycones as **1** and **2**.



14, R^1 = R_{tt}; R^2 = CO_2H
15, R^1 = R_{ct}; R^2 = CO_2H
16, R^1 = R_{tt}; R^2 = H
17, R^1 = R_{ct}; R^2 = H



18, R = H
19, R = R_{ct}

Acidic hydrolysis of the second tumor inhibitor, saponin Q, gave a complex mixture of aglycones, which was fractionated by tlc. Most of the fractions were still mixtures but one major fraction, aglycone B, was homogeneous and was studied further. The molecular ion in the mass spectrum corresponded to $C_{39}H_{60}O_7$ (m/e 640). The ultraviolet spectrum, λ_{max} 262 mμ, suggested the presence of a diene ester group and the mass spectrum contained ions at m/e 137 and 109 characteristic of a C_9-diene ester function. Signals in the nmr spectrum at τ 4.20 (d, J = 11 Hz, α H) and 3.39 (t, J = 11 Hz, β H) were comparable to those in the spectrum of **2** and suggested the probable presence of a 2-cis-4-trans acyl group. There was no signal attributable to an acetyl group.

Alkaline hydrolysis of aglycone B yielded an acidic sapogenin, $C_{30}H_{48}O_6$, which was also isolated from the products of successive acidic and alkaline hydrolysis of saponin Q. Elemental analysis and mass spectroscopy showed the presence of an additional oxygen compared to acerogenic acid. Methylation using ethereal diazomethane gave a methyl ester, $C_{31}H_{50}O_6$. On acetylation the methyl ester yielded a methyl ester tetraacetate, $C_{39}H_{58}O_{10}$, and thus the additional oxygen could be assigned to a fourth hydroxyl group.

The mass spectra of these three compounds were studied. Peaks at m/e 280, 294, and 378 in acid, ester, and peracetyl ester, respectively, could be assigned to the D,E ring system.[9] They appeared at the same mass and showed the same fragmentations as ions in the spectra of acerogenic acid, its methyl ester, and triacetyl methyl ester.

A smaller peak at m/e 224 in the spectrum of the methyl ester was assignable to the A,B ring system and appeared 17 mass units higher than in the spectrum of **5**. Thus the D,E ring system could be assumed to have the same substituents as acerogenic acid and the additional hydroxyl group could be assigned to the A,B ring system.

The nmr spectrum of the tetraacetyl methyl ester was very similar to the spectrum of **6**. It contained signals at τ 4.82 and 5.05 (AB quartet, J = 10.8 Hz) and at τ 5.43 (bt) assignable to a C-21,22 diacetate and a C-3 (CHOH) proton, respectively. In addition, it contained a second AB quartet (J = 11.5 Hz) at τ 5.65 and 5.89, which could be assigned to an axial $-CH_2OAc$ system at either C-24 or C-25.[20]

Treatment of the methyl ester with acetone and perchloric acid yielded an acetonide, $C_{34}H_{54}O_6$. Sapogenin B was therefore proposed to be 24-hydroxyacerogenic acid (**18**) and the aglycone B could be postulated to be the C-21 2-*cis*-4-*trans*-diene ester **19**. It is possible that in saponin Q the nucleus was acetylated at C-22, in a similar way to **1** and **2** and that **19** was an artefact formed by selective deacetylation under the acidic conditions of the aglycone formation reaction.

Direct alkaline hydrolysis of saponin P yielded after methylation the diene esters **11** and **12** in a 1:1.5 ratio, similar to the estimated ratio of **1** and **2** present in the

(20) A. Gaudemer, J. Polonsky, and E. Wenkert, *Bull. Soc. Chim. Fr.*, 407 (1964); M. Shamma, R. E. Glick, and R. O. Mumma, *J. Org. Chem.*, **27**, 4512 (1962).

acid hydrolysate. Hydrolysis of saponin Q, followed by methylation, also yielded the same diene esters as the main volatile components.

As the presence of α,β-unsaturated carbonyl functions has been shown to be responsible for the tumor inhibitory activity of other natural products,[21,22] the unsaturated esters in saponin P may make a highly significant contribution in making the compound the most promising of the known tumor-inhibitory saponins.[23]

Experimental Section

Infrared spectra were measured on a Beckman IR-9 or Perkin-Elmer 257 spectrometer and ultraviolet spectra were measured on a Coleman-Hitachi EPS-3T or Beckman DK-2A spectrometer in methanol solutions. CD spectra were measured using a modified JASCO spectrometer. Melting points were determined using a Thomas-Hoover melting point apparatus. Nmr spectra were recorded on a Varian A-60A or HA-100 instrument using TMS as reference as $CDCl_3$ solutions unless otherwise stated. Mass spectra were measured on an AEI MS902 high-resolution instrument or a Hitachi Perkin-Elmer RMU-6E low-resolution instrument. Vpc was carried out on a Varian Aerograph 1760 instrument using either (a) 8% QF1, 9 ft × 1/8 in. at 100° or (b) 10% Carbowax 20M, 6 ft × 1/8 in. at 100°, and the retention times are expressed relative to methyl decanoate (R_{t10}). Vpc of sugars was carried out on a F & M Model 700 chromatograph using a 10% SE-30 column at 160–240°. Tlc was carried out on precoated tlc plates of silica gel F-254 or aluminum oxide F-254 (E. Merck). Analyses were carried out by Spang microanalytical service, Ann Arbor, Mich.

Acerotin (1) and Acerocin (2).—A solution of saponin P (1.0 g) in EtOH (25 ml) and 2 *N* HCl (25 ml) was heated at 100° for 80 min. The mixture was extracted with chloroform (total 100 ml), which was washed with 75% aqueous EtOH, dried, and evaporated. The residue was separated by tlc on silica gel ($CHCl_3$–EtOH 10:1). The major band was extracted and separated by tlc on alumina (H_2O-saturated butanone).

After acidification the upper band was extracted with butanone to give a residual oil. Repeating the separation on silica gel and then on alumina yielded a gum, which on crystallization from aqueous EtOH yielded acerotin (1, 14 mg): R_f (alumina, H_2O-saturated butanone) 0.57; mp 240–243°; $[\alpha]^{24}D$ 67° (*c* 0.73, $CHCl_3$); uv max 264 mμ (ε 28,400); ir (KBr) 2.81, 2.94, 3.14, 5.73, 5.76, 5.87, 6.11, and 6.20 μ; mass spectrum *m/e* (rel intensity) 666 (M^+) (10), 466 (71), 452 (19), 407 (42), 398 (14), 368 (11), 304 (47), 276 (20), 244 (29), 216 (20), 207 (36), 199 (40), 190 (34), 137 (100), 109 (75).

Anal. Calcd for $C_{41}H_{62}O_7$: C, 73.83; H, 9.37; mol wt, 666.4496. Found: C, 73.63; H, 9.11; mol wt (mass spectrum), 666.4496.

The lower band was extracted and repurified as above to yield crystals (51 mg), which on crystallization twice from aqueous EtOH gave acerocin (2): R_f (alumina, H_2O-saturated butanone) 0.55; mp 205–207°; $[\alpha]^{24}D$ 104° (*c* 0.94, $CHCl_3$); uv max 266 mμ (ε 22,900); ir (KBr) 2.78, 2.92, 3.14, 5.71, 5.77, 5.81, 6.12, and 6.26 μ; mass spectrum *m/e* (rel intensity) 666 (19), 512 (11), 466 (87), 452 (20), 424 (17), 407 (38), 398 (10), 368 (18), 304 (48), 276 (19), 244 (27), 216 (13), 207 (43), 199 (33), 190 (37), 137 (100), 109 (94).

Anal. Calcd for $C_{41}H_{62}O_7$: C, 73.83; H, 9.37; mol wt, 666.4496. Found: C, 74.04; H, 9.46; mol wt (mass spectrum), 666.4513.

Sugars from Saponin P.—Saponin P was hydrolyzed as above but using 1 *N* HCl and the acidified aqueous solution was extracted with chloroform. The aqueous solution was evaporated and the residue, after trimethylsilylation[24] was examined by vpc. By comparison with standards, which had been treated under the same hydrolysis conditions, it was shown that saponin P had yielded arabinose and glucose in a 1:4 ratio.

Hydrolysis of Acerotin (1).—A solution of acerotin (10 mg) in 5% methanolic KOH (1 ml) was heated on a steam bath for 1 hr under nitrogen. The solution was concentrated, acidified, and extracted with hexane (two 2-ml portions) which was washed and evaporated to give an oil (2.0 mg). The oil was treated with ethereal diazomethane to give a solution which was separated by vpc (column a). The major component, which was trapped as a $CDCl_3$ solution, was the diene ester 11: R_{t10} (column a) 1.20; R_{t10} (column b) 1.49; CD λ_{max} 260 mμ ($\Delta\epsilon$ 2.7) [assuming ε (uv) 28,500[15]]; uv max 260 mμ; ir ($CDCl_3$) 5.87, 6.10, 6.19, and 10.00 μ; mass spectrum (vpc inlet, column a) *m/e* 168 (M^+), 139, 111, 79.

The aqueous solutions were combined and extracted with $CHCl_3$–MeOH which was washed with aqueous MeOH and evaporated to give a solid. The solid was separated by tlc on silica gel (H_2O-saturated butanone) to give, after crystallization from aqueous MeOH, acerogenic acid (3, 6.7 mg): R_f 0.42; mp 308–310°; $[\alpha]^{24}D$ 66° (*c* 0.95, EtOH); CD λ 222 mμ ($\Delta\epsilon$ −1.48); uv end absorption 210 mμ (ε 4400); ir (KBr) 2.92, 5.90 μ.

Anal. Calcd for $C_{30}H_{48}O_5$: C, 73.73; H, 9.90; mol wt, 488. Found: C, 73.87; H, 10.06; mol wt (mass spectrum), 488.

Hydrolysis of Acerocin (2).—Hydrolysis of acerocin (2, 10 mg) by the same method as 1 gave acerogenic acid (3, 7.1 mg), identical with 3 from 1 on comparison by melting point, mixture melting point, tlc, and ir and mass spectra, and the volatile diene ester 12 (as $CDCl_3$ solution): R_{t10} (column a) 0.57; R_{t10} (column b) 1.00; CD λ_{max} 263 mμ ($\Delta\epsilon$ 1.5) [assuming ε (uv) 23,800[15]]; uv max 263 mμ; ir ($CDCl_3$) 5.87, 6.13, 6.26, 10.10, and 10.42 μ.

Bromo-γ-lactone 13.—A solution of 3 (26 mg) in methanol (2 ml) was treated with a solution of bromine (10 mg) in methanol (1 ml). After 10 min the solution was concentrated and cooled to give crystals (26 mg). Recrystallization from methanol gave the bromolactone 13: mp 240–241° dec; $[\alpha]^{24}D$ 69° (*c* 0.59, EtOH); ir (KBr) 5.66 μ.

Anal. Calcd for $C_{30}H_{47}BrO_5 \cdot H_2O$: C, 61.52; H, 8.43; Br, 13.64; mol wt, 567. Found: C, 61.31; H, 8.31; Br, 13.62; mol wt (mass spectrum), 568 and 566.

Triacetylacerogenic Acid (4).—A solution of acerogenic acid (25 mg) in acetic anhydride (0.25 ml) and pyridine (0.25 ml) was heated for 1 hr on a steam bath. Working up in the normal way gave the crude product which was separated by tlc on silica gel ($CHCl_3$–EtOH 10:1) to give a gum (27 mg). Crystallization from acetonitrile yielded 4 (17 mg): mp 296–297° dec; $[\alpha]^{24}D$ 60° (*c* 0.81, $CHCl_3$); ir (KBr) 5.71 and 5.87 μ.

Anal. Calcd for $C_{36}H_{54}O_8$: C, 70.33; H, 8.85; mol wt, 614. Found: C, 70.44; H, 8.81; mol wt (mass spectrum), 614.

Methyl Acerogenate (5).—A solution of acerogenic acid (3, 11 mg) in MeOH (2 ml) was treated with an excess of ethereal diazomethane. The solvent was evaporated and the residue separated by tlc on silica gel ($CHCl_3$–EtOH 10:3) to give a solid, which on recrystallization from methanol yielded 5 (10 mg): mp 236–238°; $[\alpha]^{24}D$ 67° (*c* 1.01, EtOH); ir (KBr) 5.82 μ.

Anal. Calcd for $C_{31}H_{50}O_6$: C, 74.06; H, 10.03; mol wt, 502. Found: C, 73.93; H, 9.96; mol wt (mass spectrum), 502.

Methyl Triacetylacerogenate (6).—A solution of 5 (31 mg) in acetic anhydride (0.5 ml) and pyridine (0.5 ml) was kept at room temperature for 24 hr. Working up the reaction yielded a resin (40 mg) which was crystallized from methanol to give 6 (24 mg): mp 212–213°; $[\alpha]^{24}D$ 54° (*c* 1.08, $CHCl_3$); ir (KBr) 5.69, 5.77, and 8.04 μ.

Anal. Calcd for $C_{37}H_{56}O_8$: C, 70.67; H, 8.98; mol wt, 628. Found: C, 70.78; H, 8.95; mol wt (mass spectrum), 568 (M^+ − 60).

Reduction of 5.—A solution of 5 (103 mg) in THF (20 ml) was refluxed with lithium aluminum hydride (250 mg) for 20 hr. Wet THF was added and the solution was filtered. The solid was acidified and extracted with $CHCl_3$–EtOH (10:1) and the combined organic solutions were evaporated. The residue was separated by tlc on silica gel ($CHCl_3$–EtOH 10:1) to give crystals (98 mg). Recrystallization from ethanol yielded the tetrol 7, mp 298–301°.

Anal. Calcd for $C_{30}H_{50}O_4$: C, 75.90; H, 10.62; mol wt, 474. Found: C, 76.01; H, 10.69; mol wt (mass spectrum), 474.

The product was identical with 16-deoxybarringtogenol C on the basis of direct comparison[12] by mixture melting point, mixture tlc, and mass and infrared spectra. (The ir spectral comparison was kindly carried out by Professor Yosioka.)

(21) S. M. Kupchan, *Trans. N. Y. Acad. Sci.*, **32**, 85 (1970).

(22) S. M. Kupchan, D. C. Fessler, M. A. Eakin, and T. J. Giacobbe, *Science*, **168**, 376 (1970).

(23) J. L. Hartwell and B. J. Abbott, *Advan. Pharmacol.*, **7**, 117 (1969).

(24) C. C. Sweeley, R. Bentley, M. Makita, and W. W. Wells, *J. Amer. Chem. Soc.*, **85**, 2497 (1963).

Acetylation gave a tetraacetate: mp 223–224°; $[\alpha]^{24}D$ 55° (*c*, 1.10, $CHCl_3$); ir (KBr) 5.74 μ [lit.[13] mp 225–226°; $[\alpha]D$ 50° (*c* 0.8, $CHCl_3$)]. (Nmr spectra were found to be comparable by Professor Yosioka.)

Anal. Calcd for $C_{38}H_{58}O_8$: C, 70.99; H, 9.09; mol wt, 642. Found: C, 70.99; H, 9.11; mol wt (mass spectrum), 582 ($M^+ - 60$).

Deacetylacerotin (8).—A solution of acerotin (1, 13 mg) in EtOH (4 ml) and 2 *N* HCl (2 ml) was heated under reflux for 7 hr. The solution was concentrated and extracted with $CHCl_3$–EtOH. The extract was separated by tlc on silica gel ($CHCl_3$–EtOH 25:2) to give as gums 1 (6.5 mg), R_f 0.48, and **8** (3.0 mg): R_f 0.37; uv max 263 mμ (ε 27,900).

Deacetylacerocin (9).—Acerocin (2, 15 mg) was hydrolyzed in the same way as 1 to give as resins 2 (8.7 mg), R_f 0.48, and **9** (3.2 mg), R_f 0.37.

Diketone 16.—A solution of **8** (3.0 mg) in acetone (0.5 ml) was treated with 8 *N* CrO_3 in sulfuric acid at 0° for 70 min. Methanol was added and the solution was separated by tlc on silica gel ($CHCl_3$–EtOH 25:2) to give as a gum, the diketo acid **14** (2.1 mg): R_f 0.43; R_f (alumina, $CHCl_3$–EtOH 25:2) 0.01. **14** was placed on the origin of a silica gel tlc plate, which was then heated at 140° for 100 sec. The plate was developed ($CHCl_3$–EtOH 25:2) to yield as a gum, the diketone **16** (1.4 mg): R_f (silica gel, $CHCl_3$–EtOH 25:2) 0.75; R_f (alumina, $CHCl_3$–EtOH 25:2) 0.63; uv max 264 mμ (ε 25,000); ir ($CHCl_3$) 5.78, 5.88, and 6.10 μ; mass spectrum *m/e* 576, 422, 407, 394, 202, 137, 109. **1**, **2**, **8**, and **9** were all stable under the pyrolysis conditions.

Diketone 17.—**9** (3.2 mg) was oxidized in the same way as **8** to yield as a gum, the diketo acid **15** (1.9 mg): R_f (silica gel, $CHCl_3$–EtOH 25:2) 0.43; R_f (alumina, $CHCl_3$–EtOH 25:2) 0.01. Pyrolysis of **15** gave the diketone **17** (1.2 mg): R_f (silica gel, $CHCl_3$–EtOH 25:2) 0.75; R_f (alumina, $CHCl_3$–EtOH 25:2) 0.63; uv max 265 mμ (ε 17,100); ir ($CHCl_3$) 5.78, 5.88, 6.10, and 6.25 μ; mass spectrum *m/e* 576, 519, 422, 407, 394, 202, 137, 109.

Acetylacerotin (10). A. From Acerotin (1).—A solution of acerotin (9 mg) in acetic anhydride (0.2 ml) and pyridine (0.2 ml) was heated at 100° for 1 hr. Work-up followed by chromatography gave a gum **10** (8.6 mg): R_f (alumina, H_2O-saturated butanone) 0.49; R_f (silica gel, $CHCl_3$–EtOH 25:2) 0.53; uv max 264 mμ (ε 28,000); ir ($CHCl_3$) 5.74, 5.81, 5.83, 6.10, and 6.20 μ.

B. From Deacetylacerotin (8).—**8** (5.5 mg) was acetylated as above to give **10** (4.3 mg), identical by ir spectroscopy and tlc to **10** derived from **1**.

Alkaline Hydrolysis of Saponin P.—A solution of saponin P (6.6 mg) in 5% methanolic KOH was heated on a steam bath for 1 hr under nitrogen. The solution was concentrated *in vacuo*, acidified with 3 *N* HCl and extracted with hexane (4 ml), which was washed, dried, and evaporated. The residue was treated with diazomethane and the products were analyzed by vpc. The major components were **11** and **12** in a 1:1.5 ratio.

Hydrolysis of Saponin Q.—Saponin Q was hydrolyzed in the same way as saponin P. Vpc showed the presence of both **11** and **12** as major components.

Aglycone B.—A solution of saponin Q (250 mg) in EtOH (5 ml) and 2 *N* HCl (5 ml) was heated on a steam bath for 7 hr. The solution was diluted with water and extracted with chloroform, which was evaporated to yield a complex mixture of products (176 mg). The mixture was separated by tlc on silica gel ($CHCl_3$–EtOH 9:1) to yield as a homogeneous gum, aglycone B (12.9 mg): R_f 0.25; mp 190–196° dec; uv max 262 mμ (ε 25,300); ir (KBr) 2.9, 5.85, and 6.10 μ; mass spectrum *m/e* 640 (M^+, $C_{39}H_{60}O_7$), 424, 398, 137 (base peak), 109.

Sapogenin B (18). A. From Aglycone B.—A solution of aglycone B (7.2 mg) in 5% KOH in MeOH (2 ml) was refluxed for 1 hr. After concentration the solution was diluted with water acidified, and extracted with $CHCl_3$–EtOH. The organic layer was washed with water and evaporated to yield a solid (9.8 mg) which on tlc (silica gel, H_2O-saturated butanone) showed two spots. The upper spot absorbed uv light and corresponded to a C_9-unsaturated acid and the lower spot, R_f 0.60, was identical with that of sapogenin B.

On treatment with diazomethane the solid yielded sapogenin B methyl ester identical by tlc and ir spectroscopy with authentic material (see below).

B. From Saponin Q.—A solution of saponin Q (475 mg) in EtOH (10 ml) and 2 *N* HCl (10 ml) was refluxed for 6.5 hr. The solution was concentrated and partitioned between 1-butanol (20 ml) and water. The butanol-soluble fraction was dissolved in 5% KOH in MeOH (20 ml) and the solution refluxed for 1 hr under nitrogen. The solution was concentrated, diluted with water, acidified with 6 *N* HCl, and washed with petroleum ether. The aqueous solution was extracted with 1-butanol which was washed and evaporated to yield a resin. The resin was separated by tlc on silica gel (H_2O–saturated butanone) to give crude product (40 mg). Crystallization from aqueous MeOH and from methanol yielded sapogenin B (**18**): R_f 0.44; mp 341.5–342° dec; $[\alpha]^{24}D$ 70° (*c* 0.87, EtOH); uv end absorption 210 mμ (ε 4090); ir (KBr) 2.9 and 5.90 μ; mass spectrum *m/e* 504 (M^+), 486, 468, 458, 440, 424, 409, 391, 280, 262, 244, 234, 224, 217, 216.

Anal. Calcd for $C_{30}H_{48}O_6$: C, 71.39; H, 9.59. Found: C, 71.32; H, 9.58.

Sapogenin B Methyl Ester.—Sapogenin B (57 mg) was methylated using ethereal diazomethane and the product was purified by tlc on silica gel ($CHCl_3$–EtOH 10:1) to yield, after crystallization twice from aqueous methanol, the methyl ester: R_f 0.30; mp 228–229°; $[\alpha]^{24}D$ 68° (*c* 0.89, EtOH); ir (KBr) 2.9 and 5.86 μ; mass spectrum *m/e* 518 (M^+) 458, 294, 276, 234, 224, 217.

Anal. Calcd for $C_{31}H_{50}O_6 \cdot 0.5\ H_2O$: C, 70.55; H, 9.76. Found: C, 70.68; H, 9.54.

Sapogenin B Tetraacetyl Methyl Ester.—A solution of sapogenin B methyl ester (18 mg) in acetic anhydride (0.6 ml) and pyridine (0.6 ml) was kept at room temperature overnight. After heating at 100° for 30 min the solvent was evaporated. The residue was separated by tlc on silica gel ($CHCl_3$–MeOH 25:1). The major product was crystallized from methanol to yield the methyl ester tetraacetate (17 mg): R_f 0.6; mp 230–232°; $[\alpha]^{24}D$ 48° (*c* 0.73, $CHCl_3$); ir (KBr) 5.71, 5.75, and 5.77 μ.

Anal. Calcd for $C_{39}H_{58}O_{10}$: C, 68.19; H, 8.51; mol wt, 686. Found: C, 68.25; H, 8.56; mol wt (mass spectrum), 686.

Acetonide of Sapogenin B Methyl Ester.—A solution of sapogenin B methyl ester (9.8 mg) in acetone (1 ml) was treated with 70% perchloric acid (1 drop) and left at room temperature for 6 hr. The solution was basified with 5% aqueous $NaHCO_3$ and evaporated. The residue was extracted with chloroform which on evaporation yielded a gum. Separation by tlc on silica gel ($CHCl_3$–EtOH 10:1) yielded an amorphous acetonide (5.1 mg): ir ($CHCl_3$) 2.80, 5.80, 5.86, and 6.25 μ; mass spectrum *m/e* 558, 530, 500, 470, 441, 294, 276, 258, 244, 234, 217.

Anal. Calcd for $C_{34}H_{54}O_6$: mol wt, 558.3920. Found: mol wt (mass spectrum), 558.3927.

Registry No.—**1**, 29038-22-0; **2**, 29038-41-3; **3**, 29038-42-4; **4**, 29168-37-4; **5**, 29038-43-5; **6**, 29206-67-5; **8**, 29246-41-1; **10**, 29168-40-9; **11**, 29038-44-6; **12**, 29038-45-7; **13**, 29168-43-2; **14**, 29246-42-2; **15**, 29168-44-3; **16**, 29168-45-4; **17**, 29168-46-5; **18**, 29168-47-6; **18** methyl ester, 29246-43-3; **18** tetraacetyl methyl ester, 29168-48-7; **18** acetonide methyl ester, 29246-44-4; **19**, 29168-49-8.

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