Bryocoumaric Acid, a New Triterpene from *Bryonia dioica*

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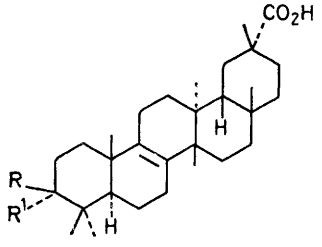
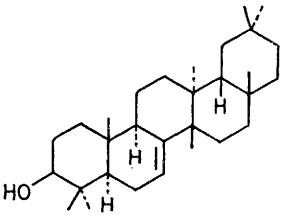
Bryocoumaric acid, from the roots of *Bryonia dioica*, is shown on the basis of chemical and spectroscopic evidence to have the structure 3α-*p*-hydroxycinnamyloxymultiflora-7,9(11)-dien-29α-oic acid. The structure of the new terpenoid portion, 3α-hydroxymultiflora-7,9(11)-dien-29α-oic acid, is confirmed by correlation with bryonolic acid, 3β-hydroxymultiflor-8-en-29α-oic acid, isolated from the same plant.

THE Cucurbitaceae contain a class of anti-tumour triterpenes with a unique carbon skeleton, the cucurbitacins,[1] as well as various triterpene acids such as echinocystic acid.[2] Thus, white bryony, *Bryonia dioica* Jacq., has been shown to possess several cucurbitacins, including cucurbitacins B, D, E, I, tetrahydro-I, J, K, L, and S,[1,3,4] and other pentacyclic triterpene acid, bryonolic acid (1),[5] which is related to multiflorenol (2).[6] In



(1) R = OH; R^1 = H
(10) R,R^1 = =O
(11) R = H; R^1 = OAc
(12) R = H; R^1 = OH



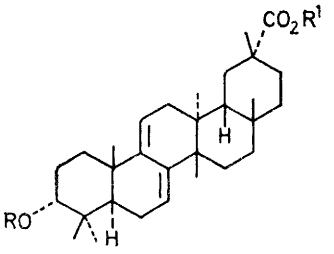
(2)

continuing our work on bryony,[4,7] we now report the isolation and structural elucidation of a new triterpene acid ester from this plant.

RESULTS AND DISCUSSION

A methanolic extract of *Bryonia dioica* roots was hydrolysed by refluxing with 2N HCl in methanol in the usual way. Extraction with chloroform and chromatography of the residue over silica gel allowed isolation, in addition to known substances, of bryocoumaric acid (3) in 0.004% yield. Mass spectrometry of (3) showed a molecular ion peak at *m/e* 600, corresponding to $C_{39}H_{52}O_5$. The u.v. spectrum showed characteristically strong absorption at 315 nm. Two pairs of doublets (δ 7.60 and 6.25, 1 H each, *J* 16 Hz and δ 7.37 and 6.80, 2 H each, *J* 9 Hz) were present in the ^{1}H n.m.r. spectrum. Both features are indicative of a *p*-substituted-cinnamyl function. Acetylation of another sample of crude extract allowed isolation, after chromatography, of two acetates, (4) and (5). The mass spectrum of (5) had a molecular ion at *m/e* 656 while that of (4) showed M^+ at *m/e* 642. The ^{1}H n.m.r. spectrum of (5) showed a sharp 3-proton singlet at δ 3.60 indicating the presence of a CO_2Me group in (5). Compound (4) was also obtained after acetylation of (3), under the usual conditions, indicating that (3) contained a hydroxy-function. However, saponification of (4) or (5) did not yield (3) but gave, as the major product, compound (6) which lacked aromatic signals in the ^{1}H n.m.r. spectrum and in the m.s. showed a molecular ion peak at *m/e* 454, corresponding with $C_{30}H_{46}O_3$. This material could also be obtained by hydrolysis with KOH in ethanol of bryocoumaric acid (3) itself, showing that (3) was the ester of an aromatic acid and a triterpene alcohol. Another product (7), isolated by chromatography of the mother-liquors of crystallisation of (6) after acetylation in the usual way, did not show the features of an acid, but gave ^{1}H n.m.r. signals characteristic of an ethyl ester. Purification by sublimation of the organic-solvent insoluble fraction of the saponification product of (3) allowed isolation of a material identical with *p*-hydroxycinnamic acid, indicating the constitution of the esterifying acid and confirming deductions from the ^{1}H n.m.r. and u.v. spectra of (3).

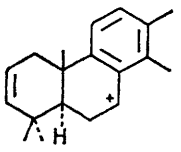
The hydrolysis product (6) readily formed a monoacetate derivative (8), similar to the acetate (7) of the minor product, on treatment with acetic anhydride–pyridine. The more polar acetate (8) showed a molecular ion peak in the mass-spectrum at *m/e* 496 (corresponding to $C_{32}H_{48}O_4$) while the less polar ethyl ester (7) had a molecular ion peak which corresponded to $C_{34}H_{50}O_4$.



(3) R = *p*—$HOC_6H_4CH\overset{t}{=}CHCO$; R^1 = H
(4) R = *p*—$AcOC_6H_4CH\overset{t}{=}CHCO$; R^1 = H
(5) R = *p*—$AcOC_6H_4CH\overset{t}{=}CHCO$; R^1 = Me
(6) R = H; R^1 = H
(7) R = Ac; R^1 = Et
(8) R = Ac; R^1 = H

Prominent peaks corresponding with loss of side-chain fragments were not present in the mass spectrum of either (7) or (8), indicating that the compounds were probably pentacyclic.[8] The mass-spectral breakdown patterns were not similar to those of oleanane, ursane, or

lupane derivatives,[9] but each showed an intense peak at *m/e* 253 [shown by accurate mass measurement to correspond to (9)] indicating either a baurenene or multiflorene-type skeleton,[10] *i.e.* a compound with methyl substituents at positions 13 and 14. However the ^{1}H n.m.r. spectra of (7) and (8) each showed seven skeletal methyl group signals all as singlets, so eliminating the baurene skeleton for which two methyl *doublets* would have been observed.



m/e 253

(9)

The u.v. spectra of (7) and (8) were almost superimposable: that of (7) showed maxima at 233, 240, and 248 nm [(8) gave 234, 241, and 249 nm] indicating the likely presence of a conjugated heteroannular diene chromophore such as a 7,9(11)-diene.[11,12] This suggestion was confirmed by the presence of ^{1}H n.m.r. 1 H multiplets at δ 5.21 and 5.43 (8) and δ 5.22 and 5.43 (7) (assigned to H-11 and H-7 respectively.)[13] An additional narrow multiplet ($W_{\frac{1}{2}} = 6$ Hz) at *ca.* δ 4.68 in the ^{1}H n.m.r. spectrum of each compound confirmed the presence of a $MeCO_2$–CH group, probably at C-3, but the unusually low $W_{\frac{1}{2}}$ value indicated that this substituent was α.

Thus (6) is a triterpene alcohol acid of the multiflorene type, with double bonds at 7 and 9(11) and a 3α-hydroxy-group. The final problem was the placement of the carboxy function. Since fragment (9) is formed on mass spectrometry this substituent could only be at positions 28, 29, or 30. However, also from an examination of the mass spectrum, the CO_2H function could not be at position 28 because C-28 acids and esters normally given an intense mass-spectral peak due to ready loss of these substituents.[9] The mass spectrum of (7) and (8) showed no such peak. The positions of the methyl signals in the ^{1}H n.m.r. spectra of (7) and (8) are very similar except that one signal (at either δ 1.25 or 1.22) in that of (8) experiences an upfield shift of *ca.* 0.2 p.p.m. to δ 1.02 in the spectrum of (7). Since the acid must be either at position 29 or 30, only if it is at 29 can it affect another group in this manner, *i.e.* that at 13, hence it may be deduced that the C-13 methyl resonates at δ 1.02 in the spectrum of (7) and at δ 1.22 (or 1.25) in that of (8), and also that the carboxy is in position 29. Compound (8) is thus 3α-acetoxymultiflora-7,9(11)-dien-29α-oic acid and (7) is the corresponding 29α-ethyl ester, probably formed as an artefact during saponification.

To confirm this, a sample of authentic bryonolic acid (1) isolated from the same plant source, was treated with chromium trioxide–pyridine to yield the known 3-ketone analogue, bryononic acid (10). If the structural proposal for (8) were correct, reduction of (6) (the corresponding 3α-alcohol) with lithium–liquid ammonia would provide the 3α-hydroxyisomultiflorenol derivative (12) which, after Sarret oxidation, should also give bryononic acid (10). Thus (6) was reduced with lithium–liquid ammonia in the usual way and the product proved to be a mixture of the Δ^8 and $\Delta^{7,9(11)}$ compounds. Separation was readily effected by argentation thin layer chromatography as the 3α-acetates. Saponification of the Δ^8 material, followed by Sarret oxidation, did provide bryononic acid, identical with the material prepared from bryonolic acid, so confirming the structural proposal for (8).

Further, since (8) was prepared by acetylation of the triterpene alcohol obtained on hydrolysis of bryocoumaric acid (3), and the only other product was *p*-hydroxycinnamic acid, the structure of (3) must be 3α-*p*-hydroxycinnamyloxymultiflora-7,9(11)-dien-29α-oic acid. Compound (5) must be the C-29 methyl ester of the acetoxybryocoumaric acid (4), probably formed as an artefact during the initial acid hydrolysis of the crude plant extract which was carried out in methanol.

Such esterified compounds are fairly unusual in nature but jacoumaric acid (the 3β-*p*-hydroxycinnamyloxy-derivative of 2α,3β-dihydroxyurs-12-en-28-oic acid) has been isolated from *Jacaranda caucana*.[14] It is also noteworthy that the present work constitutes the first report of a 3α-hydroxy-triterpene derivative in the cucurbitaceae. A preliminary note on the structure of 3α-hydroxymultiflora-7,9(11)-dien-29α-oic acid has already been published.[7]

EXPERIMENTAL

Roots of *Bryonia dioica* were collected from the Royal Botanic Gardens, Kew, London in June 1976. Dried powdered material (2.50 kg) was exhaustively extracted with methanol. Solvent was evaporated to give a black oily residue which was refluxed for 2 h with 2N HCl in methanol. This was concentrated slightly and then, after neutralisation with sodium hydrogencarbonate, was diluted with a large volume of water. Extraction into $CHCl_3$ gave a black oil (61.5 g) which was chromatographed over silica gel G.

Elution with 4% ethyl acetate–toluene gave, in addition to known substances, *bryocoumaric acid* (3) (3α-p-*hydroxycinnamyloxymultiflora*-7,9(11)-*dien*-29α-*oic acid*, which was obtained from ethyl acetate as a colourless foam (100.4 mg), m.p. 143—145 °C; $\lambda_{max.}$(EtOH) (log ε) 233 (4.18), 239 (4.17), 249 (sh) (3.89), 300 (4.13), and 315 nm (4.19); $\nu_{max.}$(Nujol) 3 400, 1 690, 1 630, and 1 605 cm^{-1}; δ($CDCl_3$) 0.78 (3 H, s), 0.85 (3 H, s), 0.94 (3 H, s), 0.97 (3 H, s), 1.01 (3 H, s), 1.22 (3 H, s), 1.24 (3 H, s), 4.80 (1 H, br s, $W_{\frac{1}{2}}$ 6 Hz), 5.20 (1 H, br m), 5.41 (1 H, br m), 6.25 (1 H, d, *J* 16 Hz), 6.80 (2 H, d, *J* 9 Hz), 7.60 (1 H, d, *J* 16 Hz), and 7.37 (2 H, d, *J* 9 Hz); *m/e*: 600 (50%), 554 (14), 539 (11), 436 (59), 423 (10), 422 (34), 421 (100), 375 (11), 254 (18), 253 (41), 248 (23), 242 (13), 241 (13), 239 (18), 235 (13), 227 (42), 225 (17), 214 (13), and 213 (21) (Found: M^+, 600.3815. $C_{39}H_{52}O_5$ requires *M*, 600.3788).

Acetylation of (3) with acetic anhydride–pyridine in the usual way gave (4) 3α-p-*acetoxycinnamyloxymultiflora*-

7,9(11)-*dien*-29α-*oic acid*, isolated as colourless needles from methanol, m.p. 158—160 °C; u.v.: $\lambda_{max.}$(EtOH) (log ε) 232 (4.44), 241 (4.50), 248 (4.41), and 283 nm (4.62); i.r.: $\nu_{max.}$(Nujol) 1 760, 1 705, 1 635, and 1 600 cm^{-1}; δ($CDCl_3$) 0.78 (3 H, s), 0.86 (3 H, s), 0.89 (3 H, s), 0.94 (3 H, s), 0.96 (3 H, s), 1.04 (3 H, s), 1.21 (3 H, s), 2.28 (3 H, s), 4.85 (1 H, s, $W_{\frac{1}{2}}$ 6 Hz), 5.25 (1 H, br m), 5.46 (1 H, br m), 6.40 (1 H, d, *J* 16 Hz), 7.15 (2 H, d, *J* 9 Hz), 7.53 (2 H, d, *J* 9 Hz), and 7.66 (1 H, d, *J* 16 Hz); *m/e* 642 (18%), 641 (28), 601 (14), 600 (31), 597 (17), 596 (29), 594 (10), 436 (34), 422 (72), 421 (72), 375 (18), 368 (12), 254 (25), 253 (36), 235 (12), and 227 (28) (Found: C, 76.5; H, 8.6. $C_{41}H_{54}O_6$ requires C, 76.6; H, 8.45%).

Another batch of roots was extracted as before but acetylated in the usual way before chromatography. Purification by chromatography on silica gel G allowed isolation of acetates (4) (identical with that previously described) and (5), *methyl* 3α-p-*acetoxycinnamyloxymultiflora*-7,9(11)-*dien*-29α-*oate*, m.p. 206—208 °C; $\lambda_{max.}$(EtOH) (log ε) 226 (4.17), 231 (4.12), 240 (4.13), 249 (4.05), and 282 nm (4.23); $\nu_{max.}$(Nujol) 1 760, 1 730, 1 700, and 1 630 cm^{-1}; δ($CDCl_3$) 0.70 (3 H, s), 0.88 (6 H, s), 0.97 (3 H, s), 1.04 (6 H, s), 1.20 (3 H, s), 2.29 (3 H, s), 3.60 (3 H, s), 4.83 (1 H, br m, $W_{\frac{1}{2}}$ 6 Hz), 5.25 (1 H, br m), 5.48 (1 H, br m), 6.42 (1 H, d, *J* 16 Hz), 7.12 (2 H, d, *J* 8 Hz), 7.54 (2 H, d, *J* 8 Hz), 7.63 (1 H, d, *J* 16 Hz); *m/e* 656 (74%), 614 (12), 450 (10), 435 (33), and 253 (17) (Found: 656.4050. $C_{42}H_{56}O_6$ requires 656.4077. Found: 253.1948. $C_{19}H_{25}$ requires 253.1956).

Saponification of Bryocoumaric Acid (3).—Compound (3) (60 mg) was refluxed with 5% KOH in EtOH for 24 h. Dilution with a large volume of water and extraction into $CHCl_3$ gave a mixture of triterpenes which was separated by chromatography on silica gel G to give compound (6), 3α-*hydroxymultiflora*-7-9(11)-*dien*-19α-*oic acid* (39 mg, 86%) which crystallised as colourless needles from ethyl acetate, m.p. 204—206 °C; u.v.: $\lambda_{max.}$(EtOH) (log ε) 232 (4.04), 241 (4.10), and 248 nm (4.04); $\nu_{max.}$(Nujol) 3 500 and 1 705 cm^{-1}; δ($CDCl_3$) 0.74 (3 H, s), 0.85 (3 H, s), 0.92 (6 H, s), 1.02 (3 H, s), 1.24 (3 H, s), 1.25 (3 H, s), 3.42 (1 H, m, $W_{\frac{1}{2}}$ 6 Hz), 5.25 (1 H, br m), and 5.42 (1 H, br m); *m/e*, 454 (100%), 439 (12), 423 (11), 421 (36), 271 (16), 255 (11), 253 (17), 239 (11), 227 (20), and 213 (16) (Found: C, 79.85; H, 10.6. $C_{30}H_{46}O_3$ requires C, 79.3; H, 10.3%). Acetylation of (6) with acetic anhydride–pyridine in the usual way afforded (8), 3α-*acetoxymultiflora*-7,9(11)-*dien*-29α-*oic acid*, m.p. 267—268 °C; $\lambda_{max.}$(EtOH) (log ε) 234 (4.04), 241 (4.08), and 249 nm (3.91); $\nu_{max.}$(Nujol) 3 350—2 750, 1 735, and 1 705 cm^{-1}; δ($CDCl_3$) 0.75 (3 H, s), 0.84 (3 H, s), 0.92 (3 H, s), 0.98 (3 H, s), 1.00 (3 H, s), 1.22 (3 H, s), 1.25 (3 H, s), 2.02 (3 H, s), 4.68 (1 H, m, $W_{\frac{1}{2}}$ 6 Hz), 5.21 (1 H, br m), and 5.43 (1 H, br m); *m/e* 496 (100%), 481 (4), 436 (10), 422 (21), 421 (40), 314 (6), 313 (7), 285 (7), 255 (9), 254 (12), 253 (23), 227 (23), and 213 (17) (Found: 421.3136. $C_{29}H_{41}O_2$ requires 421.3104). Acetylation, followed by chromatography of the mother-liquors of the isolation of (6) from the saponification mixture, allowed isolation of (7), *ethyl* 3α-*acetoxymultiflora*-7,9(11)-*dien*-29α-*oate*, m.p. 216—218 °C; $\lambda_{max.}$(EtOH) (log ε) 233 (3.84), 240 (3.88), and 248 (3.66); i.r.: $\nu_{max.}$(Nujol) 1 720 and 1 260 cm^{-1}; δ($CDCl_3$) 0.68 (3 H, s), 0.83 (3 H, s), 0.85 (3 H, s), 0.92 (3 H, s), 0.97 (3 H, s), 1.02 (3 H, s), 1.17 (3 H, s), 1.24 (3 H, t, *J* 7 Hz), 2.04 (3 H, s), 4.03 (2 H, q, *J* 7 Hz), 4.67 (1 H, m, $W_{\frac{1}{2}}$ 6 Hz), 5.22 (1 H, br m), 5.43 (1 H, br m); *m/e* 524 (82%), 464 (10), 449 (36), 313 (10), 255 (11), 254 (12), 253 (24), and 227 (23). The aqueous fraction from the saponification was concentrated by evaporation and from the syrupy residue crystals of *p*-hydroxycinnamic acid, identical in every respect with an authentic specimen, were obtained by sublimation.

Oxidation of Bryonolic Acid.—Bryonolic acid (1) (50 mg) was dissolved in pyridine containing chromium trioxide (*ca.* 100 mg). The mixture was stirred at room temperature overnight. After the usual work-up, prisms of bryononic acid [5] (10) (30 mg) were obtained, m.p. 222—225 °C; i.r. $\nu_{max.}$(Nujol) 3 400, 1 725, and 1 680 cm^{-1}; δ($CDCl_3$) 0.86 (3 H, s), 0.97 (3 H, s), 1.04 (6 H, s), 1.09 (3 H, s), 1.23 (3 H, s), 1.26 (3 H, s), *m/e* 454 (15%), 439 (35), 393 (14), 258 (11), 257 (69), 246 (19), 245 (89), 236 (39), 235 (100), 221 (13), 220 (11), 219 (14), and 205 (27).

Lithium–Liquid Ammonia Reduction of (6).—Compound (6) (20 mg) was dissolved in dry ether (50 ml) and added to anhydrous ammonia (100 ml). Lithium (500 mg) and after 10 min absolute ethanol were added, and the mixture refluxed with continuous stirring for 4 h. Preliminary experiments indicated that the products could best be separated by argentation t.l.c. of the 3α-acetate derivatives. Accordingly, after removal of the solvents the crude product was acetylated in the usual way. Purification by preparative chromatography using plates impregnated with 12.5% silver nitrate, allowed isolation of (11), 3α-*acetoxymultiflor*-8-*enoic acid* (13 mg), as colourless needles from ethyl acetate, m.p. 220—222 °C; $\nu_{max.}$(Nujol) 3 000, 1 718, and 1 685 cm^{-1}; δ($CDCl_3$) 0.85 (3 H, s), 0.90 (3 H, s), 0.94 (3 H, s), 0.97 (3 H, s), 1.03 (3 H, s), 1.24 (3 H, s), 1.26 (3 H, s), 2.05 (3 H, s), and 4.69 (1 H, m, $W_{\frac{1}{2}}$ 6 Hz); *m/e* 498 (23%), 482 (19), 424 (23), 423 (54), 302 (13), 301 (43), 290 (13), 289 (17), 242 (16), 241 (87), 236 (19), 235 (29), 230 (16), 229 (67), 221 (21), 215 (10), 214 (12), 213 (10), 204 (19), and 203 (35) (Found: C, 77.1; H, 10.25. $C_{32}H_{50}O_4$ requires C, 77.1; H, 10.0%).

Conversion of (11) *into Bryononic Acid.*—Compound (12) (10 mg) was saponified with 2% KOH in EtOH by refluxing for 4 h. Usual work-up gave an oily product, which had no ester carbonyl stretching frequency in the i.r. This crude product was dissolved in anhydrous pyridine (0.5 ml) containing chromium trioxide (*ca.* 50 mg). The mixture was stirred at room temperature overnight. Usual work-up gave bryononic acid, (10) (8 mg), identical in very respect with the material prepared from bryonolic acid (1).

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