Phytochemistry, 1979. Vol. 18, pp. 1843–1845. © Pergamon Press Ltd. Printed in England. 0031-9422/79/1101-1843 $02.00/0

3α-HYDROXY-MULTIFLORA-7,9(11)-DIEN-29α-OIC ACID, A NEW TRITERPENE FROM *BRYONIA DIOICA*

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(*Received* 27 *March* 1979)

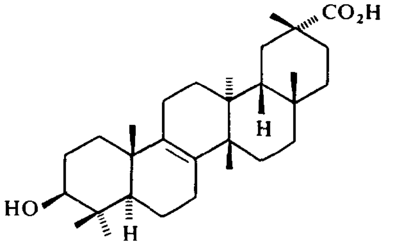
Key Word Index—*Bryonia dioica*; Cucurbitaceae; triterpene; multiflorenol; 3α-hydroxy-multiflora-7,9(11)-dien-29α-oic acid.

Abstract—The structure of a new triterpene acid from the roots of *Bryonia dioica* is shown on the basis of chemical and spectroscopic evidence to be 3α-hydroxy-multiflora-7,9(11)-dien-29α-oic acid. This is the first report of a naturally occurring $\Delta^{7,9(11)}$-multiflorene compound.

INTRODUCTION

In addition to the cucurbitacins [1], the Cucurbitaceae contain various triterpene acids. Indeed, echinocystic acid was first isolated from *Echinocystis fabacea* [2] and momordic acid (1-keto-oleanolic acid) is unique to *Momordica cochinchinensis* [3].

The white bryony, *Bryonia dioica*, has been shown to contain another pentacyclic triterpene acid, bryonolic acid (**1**) [4], which is related to multiflorenol (**2**) [5]. We now report the isolation and structural elucidation of a new triterpene acid from this plant.



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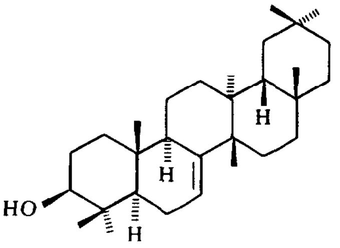

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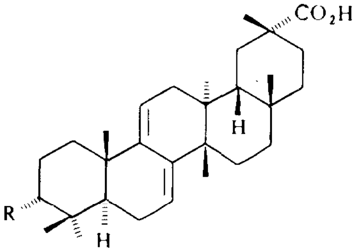
RESULTS AND DISCUSSION

A methanolic extract of *Bryonia dioica* roots was hydrolysed with 2 N HCl in methanol in the usual way and the oily product saponified with 5% KOH in ethanol to give a black residue. Repeated chromatographic separations allowed isolation of an amorphous compound which was difficult to crystallize. MS showed that this material was a mixture of two components with molecular ions at *m/e* 454 and 482, respectively. This mixture was acetylated and its constituents separated by PLC. The acetate **3** (R = OAc) of the more polar product crystallized from ethyl acetate as needles, mp 267–268°, and had a molecular ion at *m/e* 496. Accurate mass measurements showed that this corresponded with a formula of $C_{32}H_{48}O_4$. The less polar acetate **4** (R = OAc) crystallized from ethyl acetate as needles, mp 216–218° and had *m/e* 524. Prominent peaks corresponding with loss of side-chain fragments were not present in the MS of either compound, indicating that the compounds were probably pentacyclic [6]. The MS breakdown patterns of **3** (R = OAc) and **4** (R = OAc) were not similar to those of oleanane, ursane or lupane derivatives [7], but each showed an intense peak at *m/e* 253 indicating either a bauerene- or multiflorene-type skeleton [8], i.e. a compound with methyl substituents at C-13 and C-14. The ¹H NMR spectra of **3** (R = OAc)



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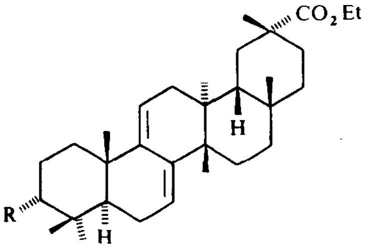

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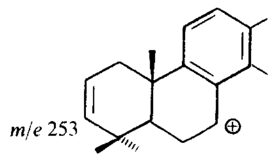

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and 4 (R = OAc) showed absorptions for seven methyl groups, all of which were singlets, so eliminating the possibility that the materials were of the bauerene type for which two methyl doublets would have been observed.

The UV spectrum of 3 (R = OAc) showed absorptions at 234, 241 and 249 nm (while that of 4 (R = OAc) showed peaks at 233, 240 and 248 nm), indicating the presence of a conjugated heteroannular diene chromophore. Such absorptions are characteristic of double bonds at positions C-7 and C-9(11) in the euphol, euphorbol and tirucallol series [9]. More specifically, the maximum at around 240 nm was indicative of 13α, 14β-methyl compounds such as multiflorenol (2) [5] and baurenol and euphol [9, 10]. This suggestion was given further weight by the presence of two 1H multiplets in the ^{1}H NMR spectrum of 3 (R = OAc) at δ 5.21 and 5.43 (the corresponding signals in the spectrum of 4 (R = OAc) were at 5.22 and 5.43). The vinyl signal at 5.21 in the spectrum of 3 (R = OAc) (5.22 in 4 (R = OAc)) was assigned to H-11 and that at 5.43 in the spectra of 3 (R = OAc) and 4 (R = OAc) to H-7 [11].

Singlets (3H) due to acetyl groups were present at δ2.02 and 2.04 in the ^{1}H NMR spectra of 3 (R = OAc) and 4 (R = OAc), respectively, but an additional narrow multiplet ($W_{\frac{1}{2}}$ = 6 Hz) at *ca* 4.68 in each spectrum was assigned to an ester methine hydrogen probably at C-3. The unusually low $W_{\frac{1}{2}}$ value of these signals indicated an axial orientation for the C-3 substituent [12], i.e. that the corresponding alcohol was 3α. The IR spectrum of 3 (R = OAc) showed bands at 3350–2750 (broad), 1735 and 1705 cm^{-1}, but in the spectrum of 4 (R = OAc), no hydroxyl absorption was present and a broad carbonyl stretching band appeared at 1720 cm^{-1}. Thus it seemed likely that 4 (R = OAc) was the artefact ester of the parent acid 3 (R = OAc). As confirmation, in the ^{1}H NMR spectrum of the ethyl ester 4 (R = OAc) a typical 2H quartet was seen at δ 4.03 and the corresponding 3H triplet was at 1.24, characteristic of an ethyl ester.

At this point it may be stated in summary that 3 (R = OAc) was the acetate of a triterpene alcohol acid of the multiflorene type, with double bonds at C-7 and C-9(11), with a 3α-hydroxyl group. Compound 4 (R = OAc) was thus the acetate of the ethyl ester of this acid. The final problem was the placing of the carboxyl group. However, since the MS of 3 (R = OAc) and 4 (R = OAc) both showed an intense peak at *m/e* 253(5) [7] (which arose by fission across ring D, proving that methyl groups must be present at C-13 and C-14), the acid could not be present at any of the positions C-4, C-10, C-13 or C-14, leaving only carbons 28, 29 or 30. C-28 may be eliminated since C-28 CO_2H or CO_2R compounds can be readily identified because they give a rather intense peak due to the ready loss of these groups compared with their corresponding C-29 or C-30 isomers [7]. The MS of 3 (R = OAc) and 4 (R = OAc) showed no such peak. Since the resonance position of the C-26 methyl group at C-13 experiences a pronounced downfield shift of *ca* 0.2 ppm (from δ 1.02 to 1.22 or 1.25) on hydrolysis of the -CO_2CH_2Me function in 4 (R = OAc) to the parent acid in 3 (R = OAc), the carboxyl group must be α, because only in this orientation can C-26 be so affected. Thus, it must be C-29 which was oxidized in 3 (R = OAc) and 4 (R = OAc). Hence the natural compound 3 (R = OH) may be formulated as 3α-hydroxy-multiflora-7,9(11)-dien-29α-oic acid and 4 (R = OAc) was the acetate of the corresponding 29α-ethyl ester, an artefact formed by *trans*-esterification during the saponification.

It is noteworthy that the title compound is similar to the other triterpene acid isolated from *Bryonia dioica*, bryonolic acid, in being a derivative of multifloren-29α-oic acid, but with a 3α-hydroxyl group. Moreover this is the first report of a naturally occurring multiflorene derivative with a 7,9(11)-diene system.

One of the products from the aqueous soluble fraction after saponification was, after purification by sublimation, shown to be *p*-hydroxy-cinnamic acid by comparison with an authentic sample. Preliminary experiments on non-saponified material have shown that this acid is combined in the plant with the 3α-hydroxy-multiflora-7,9(11)-dien-29α-oic acid as an ester. Further studies to fully characterize this material are in progress.

EXPERIMENTAL

Mps are uncorr. ^{1}H NMR spectra were determined in $CDCl_3$ soln at 90 MHz and chemical shift values are quoted in ppm downfield from TMS. as internal standard. MS were obtained on a direct inlet system operating at 70 eV. Roots of *Bryonia dioica* were collected from the Royal Botanic Gardens, Kew, London in June 1976. Dried, powdered material (2.7 kg) was exhaustively extracted with petrol bp 40–60° and then refluxed with 2 N HCl in MeOH. This was concd slightly and then saponified with 5% KOH in EtOH. Most of the solvent was removed by evapn under red. press., diluted with excess H_2O and the mixture extracted several times with $CHCl_3$. Evapn of the dried $CHCl_3$ extracts yielded a black oil (60 g) which was applied to a column of Si gel G. Elution with C_6H_6 followed by increasing concns of EtOAc in C_6H_6 allowed isolation of an amorphous compound which could not be crystallized. This was shown to be two substances by TLC (Si gel G plates, C_6H_6–EtOAc (9:1) as solvent). MS of the mixture showed two molecular ion peaks at *m/e* 454 and 482. The crude product was acetylated with Ac_2O and Py in the usual way and normal work-up gave a yellowish oil which was separated into two components on PLC (Si gel G plates, cylcohexane–EtOAc (4:1) as solvent). The more polar (major) compound crystallized from EtOAc as needles (7.6 mg) of 3α-acetoxy-multiflora-7,9(11)-dien-29-oic acid (3, R = OAc), mp 267–268°; λ_{max}^{EtOH} nm (log ε): 234 (4.04), 241 (4.08), 249 (3.91); ν_{max}^{Nujol} cm^{-1}: 3350–2750, 1735, 1705, 1720; ^{1}H NMR: δ 5.43 (1H, *m*, H-7), 5.21 (1H, *m*, H-11), 4.68 (1H, *m*, $W_{\frac{1}{2}}$ = 6 Hz, H-3β), 2.02 (3H, *s*, Me CO_2-), 1.25 (3H, *s*), 1.22 (3H, *s*), 1.00 (3H, *s*), 0.98 (3H, *s*), 0.92 (3H, *s*), 0.84 (3H, *s*), 0.75 (3H, *s*); MS *m/e* (rel. int.): 496 (100), 481 (4), 436 (10), 422 (21), 421 (40), 314 (6), 313 (7), 285 (7), 255 (9), 254 (12), 253 (23), 227 (23), 213 (17), 185 (18), 171 (20), 163 (33), 145 (25), 107 (30), 95 (43), 69 (48), 55 (50), 43 (92). Accurate mass measurement: Found: 421.3136. $C_{29}H_{41}O_2$ requires: 421.3104.

The less polar minor component also crystallized from EtOAc as needles (3.9 mg) of ethyl-3α-acetoxy-multiflora-7,9(11)-dien-29-oate (4, R = OAc), mp 216–218°; λ_{max}^{EtOH} nm (log ε): 233 (3.84), 240 (3.88), 248 (3.66); $\nu_{max}^{CHCl_3}$ cm^{-1}: 1720 (br), 1260;

^{1}H NMR: δ 5.43 (1H, *m*, H-7), 5.22 (1H, *m*, H-11), 4.67 (1H, *m*, $W_{\frac{1}{2}}$ = 6 Hz, H-3β), 4.03 (2H, *q*, J = 7 Hz, $\text{-CO}_2\text{-C}\underline{\text{H}}_2\text{-Me}$), 2.04 (3H, *s*, Me CO_2-), 1.24 (3H, *t*, J = 7 Hz, $\text{-CO}_2\text{-CH}_2\text{-}\underline{\text{Me}}$), 1.17 (3H, *s*), 1.02 (3H, *s*), 0.97 (3H, *s*), 0.92 (3H, *s*), 0.85 (3H, *s*), 0.83 (3H, *s*), 0.68 (3H, *s*); MS *m/e* (rel. int.): 524 (82), 464 (10), 449 (36), 313 (10), 255 (11), 254 (12), 253 (24), 227 (23), 163 (50), 121 (33), 119 (29), 109 (43), 107 (36), 95 (60), 81 (55), 69 (100), 55 (42), 43 (88).

The volume of the aq. layer remaining after extraction of the CHCl_3-soluble fraction was almost taken to dryness under red. pres. The viscous residue was sublimed onto a cooled microscope slide whereupon colourless crystals, mp *ca* 214° (dec.), were deposited which were identical in every respect with an authentic sample of *p*-hydroxy-cinnamic acid.

Acknowledgements—We are grateful to the Director, Royal Botanic Gardens, Kew, London, for the plant material. MS and accurate mass measurements were obtained by Mr. D. Carter, The School of Pharmacy, University of London, and Mr. G. McDonough, Chelsea College, ran the ^{1}H NMR spectra. M.T.O. thanks the University of Azarabadegan and the Government of Iran for a scholarship.

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