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A NEW TRITERPENE LACTONE FROM *GYMNOCLADUS DIOICA*

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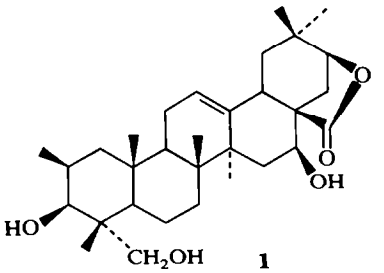
Key Word Index—*Gymnocladus dioica*; Leguminosae; new triterpene lactone; new triterpene hexol; new triterpene pentahydroxy acid; 2β,23-dihydroxyacacic acid lactone.

Abstract—2β,23-Dihydroxyacacic acid lactone was isolated from *Gymnocladus dioica* as an artifact from 2β, 3β 16β, 21β, 23-pentahydroxyolean-12-ene-28-oic acid.

Acid hydrolysis of the crude saponin from the seed pods of *Gymnocladus dioica* afforded a new triterpene lactone (**1**). The lactone was purified by chromatography on Si gel with chloroform–5% methanol and finally crystallized from chloroform–hexane and methanol–water to a colourless crystalline solid, mp 189–192°, $[\alpha]_D^{21}$ +16°. The IR spectrum exhibited strong absorption bands at 3300 cm^{-1}. (OH) and 1760 cm^{-1} (γ-lactone), while the UV spectrum showed λ_{max}^{MeOH} 260 nm (sh), ε = 395, 269 nm, ε = 412 and 278 nm (sh), ε = 374, indicating the system lacked conjugation.



1

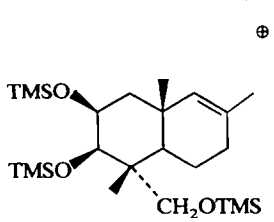
The MS of the TMS derivative showed a molecular ion at *m/e* 790, with sequential losses of three HOTMS groups at *m/e* 700, 610 and 520. The high resolution MS of the *m/e* 520 peak indicated the composition $C_{33}H_{48}O_3Si$. The molecular ion had the composition $C_{42}H_{78}O_6Si_4$, so that the parent compound should be $C_{30}H_{46}O_6$ and contain four free hydroxyl groups with the remaining two oxygens forming the lactone ring. CH analysis of **1** required the coordination of one water molecule, $C_{30}H_{46}O_6 \cdot H_2O$.

The proton coupled and decoupled ^{13}C NMR spectra, in addition to confirming the presence of thirty carbons, confirmed the presence of a carbonyl and a double bond at δ139.4 and 125 ppm shown to be associated with only one vinyl proton by the appearance of a doublet at the high field carbon in the coupled spectrum.

The five oxygenated carbons appeared at δ 84, 75, 71.1, 70.9 and 67.4 ppm. Four of these were shown to be associated with one proton by the appearance of doublets, while the fifth carbon appeared as a triplet (δ 70.9 ppm) and must be a hydroxymethyl.

Treatment of the lactone tetraacetate with selenium dioxide gave a product with a UV spectrum typical of a conjugated heteroannular diene. The molecular formula, functional groups already discussed, and the appearance of six methyl singlets in the ^1H NMR spectrum, strongly suggested the common Δ^{12}-oleanene skeleton.

The double bond at the C-12 position was readily recognized by mass spectrometry [1]. The molecular ion of the TMS derivative underwent a retro-Diels–Alder fragmentation to give the two fragments **2** and **3**. Further fragmentation of these ions accounted for some of the principal peaks in the MS (*m/e* 366, **2**-HOTMS; *m/e* 276, **2**-[2×HOTMS]; *m/e* 263, **2**-HOTMS–CH_2OTMS; *m/e* 244, **3**-HOTMS) [2]. The indicated elemental composition of each of these fragments was confirmed by high resolution MS.



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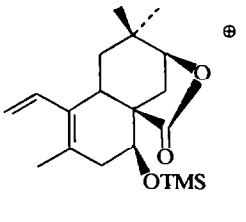

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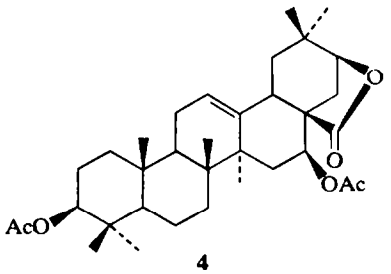
The appearance of a closely spaced triplet at δ5.40 ppm in the ^1H NMR spectrum confirmed the presence of the single vinyl proton already noted in the ^{13}C NMR spectrum. The remaining six downfield protons further confirmed the hydroxymethyl group seen as an AB (H, *d* at δ3.42, *J* = 10.8 Hz and H, *d* at 3.71 ppm, *J* = 10.8 Hz), —CHO (H, *d* at 3.62 ppm, *J* = 3.6 Hz), CHO (H, *q* at 4.00 ppm, *J* = 4.8 and 12 Hz), CHO (H, *t* at 4.11, *J* = 3.0 and 7.0 Hz) and CHO— (H, *d* at 4.24 ppm, *J* = 5.4 Hz). The doublet at 4.24 ppm remained fixed in the ^1H NMR spectrum of the tetraacetate while the other peaks shifted downfield to 3.72, 3.85, 4.92, 5.03 and 5.41 ppm respectively. This doublet was therefore associated with the lactone. Varshney [3] gives 4.22 ppm, *J* = 4 Hz and 5.0 ppm, *J* = 5 and 12 Hz for the C-21 and C-16 protons of acacic acid lactone diacetate (**4**) and he has discussed the stereochemistry of acacic acid lactone which necessitates the E-ring being constrained to a boat form and the D-ring can then adopt a twist form

in which the 16β-bond is quasi-equatorial. Varshney [3] points out that the resulting geometrical relationship between the 16α-proton and the C-15 CH_2 group explains the observed couplings. He also argues that the C-21, C-28 lactone deforms the skeleton enough to allow the hydrogenation of acacic acid lactone diacetate. The C-21, C-28 lactone of machaerinic acid has also been easily hydrogenated [4]. We have found that compound **1** fails to hydrogenate as would be expected for a typical Δ^{12}-oleanene.



4

The location of the hydroxyl at C-16 in a quasi-equatorial position explains the downfield shift of the lactone carbonyl frequency in the ^{13}C NMR spectrum from a normal 178 ppm (γ-lactone) to 181 ppm, a moderately strongly hydrogen bonded γ-lactone [5]. The carbonyl shifts back to a non-hydrogen bonded 178.7 ppm in the tetraacetate of compound **1**.

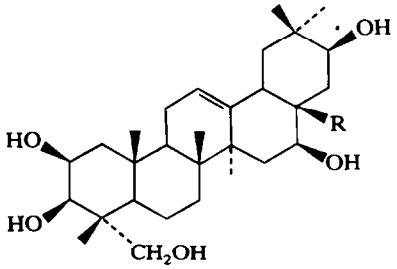
One secondary hydroxyl was assigned to C-3 primarily for biogenetic reasons. The chemical shift of the CH_2OH (and CH_2OAc) was within the range for an equatorial CH_2 group [6] and the observed loss of CH_2OTMS from **2** leaves C-23 as the only choice for this group.

The doublet at δ4.92 and multiplet at 5.41 ppm in the ^1H NMR spectrum of **1**-acetate had coupling constants and chemical shifts clearly in agreement with 2β,3β-diacetoxy (*d*, 4.88, $J = 4$, Hz and *multiplet*, 5.32 ppm, $W_{1/2} = 8$ Hz) assigned in the spectrum of jaligonic acid dimethyl ester triacetate by Woo [7].

The ^1H NMR spectrum of **1**-acetate also confirmed four acetoxy methyls at δ2.00 (3H), 2.01 (3H) and 2.06 (6H). The MS also indicated four acetates.

The chemical shifts of the methyl groups in the Δ^{12}-oleanenes with various substituents and positions on the skeleton have been intensively studied. The substituent effects have been summarized by Nakanishi [8]. Using the convenient starting compound **4** with methyl signals given at 0.89, 0.89, 0.94, 1.00, 1.00, 1.00, 1.22 ppm, we assigned the farthest downfield signal to the C-27 methyl, the two high field signals to the C-23 and C-24 methyls, and the 0.94 ppm signal to the C-25 methyl group. These assignments allowed the substituent effects to be applied to predict the methyl frequencies for compound **1** (Table 1). The close correlation of these methyl frequencies added further support to our assignments.

On treatment with acetone, dimethoxypropane and sulfuric acid at room temperature, compound **1** formed two major isopropylidene derivatives which can be separated as TMS derivatives by GLC. It has been shown that isopropylidene derivatives form between 2α-3β, 2β-3β or 3β-23 but the 3β-24 derivative formed only with more drastic conditions [9]. The fact that our two derivatives formed in about equal amounts further aids the argument. The lactone was reduced easily to give a colourless hexol **5**, mp 273–276°. The fact that isopropylidene formation was not observed between the C-16 and C-28 hydroxyl groups of the lactone hydride reduction product **5** was not surprising since these derivatives have been reported to be very unstable [10].



5 R = CH_2OH
6 R = CO_2H

The lactone **1** opened easily to the acid form (mp 307–310° decomp.) upon treatment with sodium methoxide solution and would be expected to close easily under the acidic conditions of hydrolysis.

It is assumed that the lactone was an artifact produced from **6**, the aglycone moiety found in this plant.

EXPERIMENTAL

Mps were determined on a Fisher–Johns block and are uncorr. The R_f values relate to EM Si Gel 60F-254, 0.25 mm plates and H_2SO_4 as the spray reagent. ^1H NMR spectra in $CDCl_3$ (TMS) at 360 MHz and ^{13}C NMR at 25.2 MHz.

Crude glycosides of Gymnocladus dioica. The seeds (850 g) were removed from the pods (2.5 kg) and the finely ground pods were defatted (12 g) with hexane. The ground pods (1 kg) were mixed with H_2O (1 l.) and percolated with *n*-BuOH to yield the tan glycosides (39 g) on evap.

Acid hydrolysis of crude glycosides. Crude glycosides (30 g) were refluxed in N HCl for 3 hr under N_2. A dark brown solid (12.6 g) precipitated. The ppt. was dissolved in MeOH and treated with activated C to remove the dark colour.

Table 1. ^1H NMR chemical shifts for the methyl groups of **1**

Methyls	C-23	C-24	C-25	C-26	C-27	C-29	C-30
Acacic acid lactone diacetate	0.89	0.89	0.94	1.00	1.22	1.00	1.00
2β-OAc		+0.18	+0.26	+0.03	0	0	0
23-OAc		−0.03	+0.03	0	−0.02	+0.02	+0.02
Calc. for **1**		1.04	1.23	1.03	1.20	1.02	1.02
Found		1.05	1.24	1.03	1.22	1.02	1.02

Chromatography of 4.7 g of the decolourized material on Si gel gave a main fraction (1.9 g) eluted with $CHCl_3$–MeOH (10:1). Chromatography of the main fraction on the Waters HPLC-500 (Si gel cartridge) using $CHCl_3$–MeOH (95:5) gave a fraction (125 mg) which after crystallization from $CHCl_3$–hexane and MeOH–H_2O gave colourless crystals of **1**, mp 189–192°; $[\alpha]_D^{21}+16.2°$ (*c*, 1.64 $CHCl_3$); R_f 0.42 $CHCl_3$–MeOH–HOAc (50:4:1), 0.52 cyclohexane–Me_2CO–HOAc (20:20:1); MS (TMS derivative) *m/e*: 790(M^+), 700, 610, 461.2856 ($C_{30}H_{41}O_2Si$ requires: 461.2875), 407.2419 ($C_{26}H_{35}O_2Si$ requires: 407.2406), 366.2392 ($C_{20}H_{38}O_2Si_2$ requires: 366.2411), 276.1898 ($C_{17}H_{28}OSi$ requires: 276.1909), 263.1820 ($C_{16}H_{27}OSi$ requires: 263.1831), 244.1459 ($C_{16}H_{20}O_2$ requires: 244.1463) [10]. (Found: C, 70.8; H, 9.2. $C_{30}H_{46}O_6 \cdot H_2O$ requires: C, 70.5; H, 9.5%).

Acetylation of **1**. Compound **1** (7.4 mg) acetylated with Ac_2O–Py at room temp. and purified via TLC with $CHCl_3$ gave 5.3 mg of **1**-tetraacetate as a colourless glass.

Selenium dioxide oxidation of **1**-*tetraacetate*. **1**-tetraacetate (3.6 mg) and 6.0 mg SeO_2 in 0.5 ml glacial HOAc was heated at 100° for 12 hr. The solvent was evapd, H_2O added and the products extracted into Et_2O. TLC with $CHCl_3$ separated two UV absorbing bands, the least polar, 0.5 mg, λ_{max}^{MeOH} nm: 243 (ε 13 000); 252 (ε 17 000) and 260 (ε 16 000); *m/e* 668 (M^+) was attributed to a typical heteroannulardiene. The more polar product, 1.1 mg, λ_{max}^{MeOH} nm: 237 (ε 5700), 254 (ε 10 800) and 260 (ε 10 700); *m/e* 726 (M^+), was attributed to the formation of the heteroannulardienol acetate.

Isopropylidene derivative of **1**. Compound **1** (2.5 mg) was treated for 24 hr at 20° with 1 ml of reagent (50 ml Me_2CO, 1 ml dimethoxypropane and 150 mg conc H_2SO_4). Excess $BaCO_3$ was added and stirring continued 12 hr; evapn of the filtrate gave a mixture that was separated as their TMS derivatives by GLC (OV-17 at 310°). Eight peaks were detected: 6.7% starting material-TMS; 3.6%, unknown, *m/e* − 671(M-Me), 53.8%, attributed to 2,3-isopropylidene derivative, *m/e* = 671(M-Me), 352(AB rings-C_3H_6O), 262(352-HOTMS), 244 **3** HOTMS), 204(352-C_3H_6O-HOTMS), 103(CH_2OTMS); 2.9% unknown, *m/e* = 686 (M^+); 32.9% attributed to 3,23-isopropylidene derivative, *m/e* = 686 (M^+), 671(M-Me), 628, 611, 538, 448, 407, 352, 294, 244, 204, 103 (very small); 4.1% unknown *m/e* = 686 (M^+); 1.6% unknown, *m/e* = 700 (M^+?); 2.7%, unknown, *m/e* = 614 (M^+).

Attempted hydrogenation of **1**. Hydrogenation of **1** (2.9 mg) in 0.5 ml glacial HOAc with PtO_2 at 13.5 psi and 20° for 24 hr gave starting material as the only product.

Preparation of triterpenehexol **5**. To **1** (6.5 mg) in 0.6 ml C_6H_6–THF (1:1) was added 0.3 ml 60% $NaHAl(OCH_2CH_2OMe)_2$ in C_6H_6. After 12 hr at 20°, H_2O was added and the product extracted with *n*-BuOH. The residue from evap of the *n*-BuOH was taken into Me_2CO–$CHCl_3$ and evapn gave 4.6 mg of a colourless solid, mp 273–276°. The characteristic lactone carbonyl band was missing from the IR. MS (as TMS) *m/e* 923(M-Me), 848(M-HOTMS), 758(M-2×HOTMS) 745(M-HOTMS-CH_2O-TMS), 668(M-3×HOTMS), 655(M-2×HOTMS-CH_2OTMS), 565(M-3×HOTMS-CH_2OTMS), 475(M-4×HOTMS-CH_2OTMS), 392(482-HOTMS), 366(456-HOTMS), 302-(482-2×HOTMS), 289(482-HOTMS-CH_2OTMS), 199-(482-2×HOTMS-CH_2OTMS), 103(CH_2OTMS).

Preparation of isopropylidene derivative. Compound **5** (2.3 mg) treated by the procedure described for the isopropylidene derivative of **1** gave 2.0 mg of colourless product that failed to gas chromatography as its TMS derivative. MS (on solid probe) *m/e*: 834(M), 819(M-Me), 744(M-HOTMS), 654(M-2×HOTMS), 641(M-HOTMS-CH_2OTMS), 564(M-3×HOTMS), 551(M-2×HOTMS-CH_2OTMS), 392(482-HOTMS), 302(482-2×HOTMS) 289(482-HOTMS-CH_2O-TMS), 199(482-HOTMS-CH_2OTMS), 103(CH_2OTMS).

Opening lactone ring of **1**. Compound **1** (1.6 mg) and 0.6 ml 5% NaOMe in MeOH was sealed in a glass tube under N_2 and kept at 80° for 1 hr. The mixture was neutralized with N HCl and finally $NaHCO_3$ in excess. The product was extracted with 0.3 ml *n*-BuOH. Evapn of the *n*-BuOH and crystallization from EtOH gave a colourless solid, mp 307–310° (decomp.); IR cm^{-1}: 1690 (C=O); R_f = 0.10 ($CHCl_3$–MeOH–HOAc, 50:4:1); 0.36 (cyclohexane–Me_2-CO–HOAc, 20:20:1). MS (of TMSi derivative) *m/e*: 952(M), 862(M-HOTMS), 772(M-2×HOTMS), 366(456-HOTMS), 316(496-2×HOTMS), 276(456-2×HOTMS), 263-(456-HOTMS-CH_2OTMS), 199(316-COOTMS).

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REFERENCES

1. Budzikiewicz, H., Wilson, J. M. and Djerassi, C. (1963) *J. Am. Chem. Soc.* **85,** 3688.
2. Wünsche, C. and Löw, I. (1966) *Tetrahedron* **22,** 1893.
3. Varshney, I. P., Shamsuddin, K. M. and Beyler, R. E. (1965) *Tetrahedron Letters* 1187.
4. Tursch, B., Tursch, E., Harrison, I. T., Brazão da Silva, G. B. C. T. C., Monteiro, H. J., Gilbert, B., Mors, W. B. and Djerassi, C. (1963) *J. Org. Chem.* **28,** 2390.
5. Bui, A. M., Cavé, A., Janot, M. M., Parello, J. and Potier, P. (1974) *Tetrahedron* **30,** 1327.
6. Gaudemer, A., Polonsky, J. and Wenkert, E. (1964) *Bull. Soc. Chim. Fr.* 407.
7. Woo, W. S. (1973) *Lloydia* **36,** 326.
8. Nakanishi, K., Goto, T., Ito, S., Natori, S. and Nozoe, S. (1974) *Natural Products Chemistry*, Vol. 1, p. 365. Kodausha, Tokyo and Academic Press, New York.
9. Johnson, A. and Shimizu, Y. (1974) *Tetrahedron* **30,** 2033.
10. Hensens, O. D., Khong, P. W. and Lewis, K. G. (1976) *Aust. J. Chem.* **29,** 1549.