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TRITERPENOIDS OF *TERMINALIA SERICEA**

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Key Word Index—*Terminalia sericea*; Combretaceae; triterpenoids; sericic acid; sericoside.

Abstract—The structures of sericic acid and sericoside, the major constituents of the roots of *Terminalia sericea*, have been determined. Sericic acid is 2α,3β,19α,24-tetrahydroxy-olean-12-en-28-oic acid and sericoside the corresponding C-28 *D*-glucosyl ester.

INTRODUCTION

IN THE course of our search for drugs from plant sources, an aq. MeOH extract of the roots of *Terminalia sericea* Burch. (Combretaceae) showed significant anti-inflammatory properties.[1] We report herein the fractionation of the active extract and the isolation of a new triterpene acid, named sericic acid (0·05%), which occurs together with the corresponding glycoside, sericoside (0·14%).

RESULTS AND DISCUSSION

Sericic acid (**1**), $C_{30}H_{48}O_6$, on treatment with diazomethane yielded a methyl ester (**2**). Acetylation of **2** gave a triacetate (**3**), which showed in its NMR spectrum signals attributable to a vinyl proton at δ 5·45 (1H, *m*), six tertiary C-methyl groups at δ 0·70, 0·98 (6H), 1·04, 1·08 and 1·26, three acetyl methyl groups at δ 1·98 and 2·06 (6H), a carboxy methyl group at δ 3·63, an acetoxy methylene group at δ 4·22 (2H, *s*), two protons at δ 3·34 (*d*; *J* 3 Hz) and 3·16 (*bd*, *J* 3 Hz), and one proton on a carbon carrying an acetoxyl group at δ 4·85 (*d*, *J* 11 Hz) which couples with another acetoxy methynic proton adjacent to a methylene group and resonating at δ 5·18 (*ddd*, J_1 11, J_2 10, J_3 4 Hz). In its IR spectrum **3** exhibited a band at 3640 cm^{-1} indicating incomplete acetylation. Further acetylation with acetic anhydride and perchloric acid gave a tetra-acetate (**4**), which showed no hydroxyl IR absorption and a downfield shift at δ 5·29 of the proton resonating at δ 3·16 in the NMR spectrum of **3**. Oxidation of **3** gave a ketone (**5**); these reactions indicated that one of the four hydroxyl groups in sericic acid is in a sterically hindered position. Information about the nature of the trisubstituted double bond was gained by treatment of **1** with bromine in methanol, which gave a bromo-γ-lactone (**6**), which showed an IR band at 1760 cm^{-1} and the absence of an olefinic proton signal in the NMR spectrum.

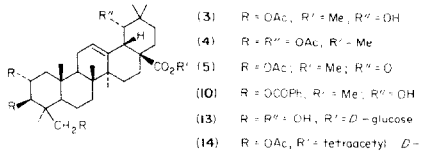
The MS of **1** exhibited the typical retro Diels–Alder fragmentation of the C-ring characteristic of the Δ^{12}-β-amyrin skeleton.[2] The strong peaks at *m/e* 264, 146 (264^+-H_2O), 219 (264^+-CO_2H) and 201 ($264^+-CO_2H-H_2O$) indicated the presence of a hydroxyl group and

* Part VII in the series "Plants of Mozambique". For Part IV see BOMBARDELLI, E., GABETTA, B. and MUSTICH, G. (1974) *Fitoterapia* **45**, 4.

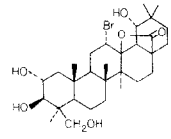
[1] BRIT. PAT. APPL. 4042/74.

[2] BUDZIKIEWICZ, H., WILSON, J. M. and DJERASSI, C. (1963) *J. Am. Chem. Soc.* **85**, 3688.

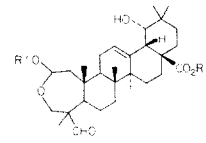
a carboxyl group in the D,E ring system; the significant peak at *m/e* 240 was attributed to an A,B ring system carrying three hydroxyl groups. The carboxyl group was assigned to the C-17 position on the basis of the CD curve of **1** which was very similar to a series of $\Delta^{1,2}$-triterpene-28-carboxylic acids[3] [λ 217 nm ($\Delta\epsilon$ — 5·2), MeOH]. This assignment was also supported by the formation of **6** after treatment of **1** with bromine.



(1) R = OH, R′ = H, R″ = OH
(2) R = OH, R′ = Me, R″ = OH
(3) R = OAc, R′ = Me, R″ = OH
(4) R = R″ = OAc, R′ = Me
(5) R = OAc, R′ = Me; R″ = O
(10) R = OCOPh, R′ = Me, R″ = OH
(13) R = R″ = OH, R′ = D-glucose
(14) R = OAc, R′ = tetraacetyl D-glucose, R″ = OH

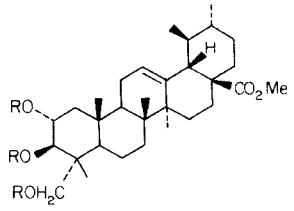


(6)

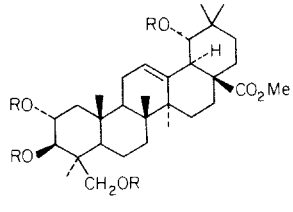


(7) R = R′ = H
(8) R = Me, R′ = H
(9) R = Me, R′ = Ac

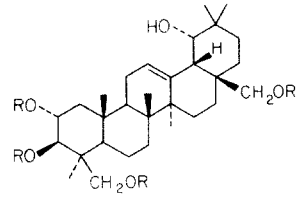
From the NMR spectrum of the triacetate **3**, two hydroxyl groups in the A,B ring system could be formulated as a 1,2 or 2,3 diequatorial diol, as the coupling (J 11 Hz) between the protons on the carbon atoms carrying the oxygen suggested a diaxial relationship with no other possibility for the location of the =C CHOH CHOH CH_2 system. Sodium periodate oxidation of **1** afforded the acid **7**, which was converted with diazomethane into the methyl ester **8** and, after acetylation, into the derivative **9**. The NMR spectrum of **9** exhibited no $-CH_2OAc$ signal, only *one* aldehydic proton as a singlet at δ 9·35, an acetyl methyl group at δ 2·05, two geminal protons as doublets (J 12 Hz) at δ 4·00 and 3·20, and a strongly deshielded axial proton (δ 6·00, *dd*) whose signal is split by an equatorial (J 5 Hz) and an axial (J 9 Hz) coupling. These data can be explained if a seven-membered A ring carrying a hemiacetal grouping is assigned to compound **9**,[4] that is locating the diequatorial diol group of **1** at the C-2,3 position and the primary alcoholic function at either C-23 or C-24. However, the absence in the NMR spectrum of **3** of a C-24 methyl group signal[5] and the chemical shift (δ 4·22) of the $-\underline{CH_2}OAc$ system showed that the primary hydroxyl group was at C-24 (an equatorial $-\underline{CH_2}OAc$ system resonates[6] at higher field than δ 3·9).



(11) R = COPh



(12) R = Ac



(15) R = H
(16) R = Ac

Independent evidence for the location of the hydroxymethyl group was obtained by comparison of the CD curves[7] of sericic acid tribenzoate methyl ester (**10**) and asiatic acid tribenzoate methyl ester (**11**). The shape of the curves in dioxane [maxima of similar intensity but opposite sign at λ 237 nm ($\Delta\epsilon$ — 12·4) for **10** and λ 235 nm ($\Delta\epsilon$ + 7·8) for **11**] indicated an opposite configuration at the C-4 centre.

[3] RENWICK, J. D., SCOPES, P. M. and HUNECK, S. (1969) *J. Chem. Soc. C*, 2544.
[4] SINGH, B. and RASTOGI, R. P. (1969) *Phytochemistry* **8**, 917.
[5] CHEUNG, H. T. and WILLIAMSON, D. G. (1969) *Tetrahedron* **25**, 119.
[6] GAUDEMER, A., POLONSKY, J. and WENKERT, E. (1964) *Bull. Soc. Chim. Fr.*, 407.
[7] HARADA, N. and NAKANISHI, K. (1969) *J. Am. Chem. Soc.* **91**, 3989.

The NMR spectrum (C_6D_6) of the tetra-acetate (**4**) had signals for acetyl methyl groups at δ 2·04 (3H) and 1·91 (9H), for the C-2, C-3 and C-24 protons at δ 5·34, 5·09 and 4·38 respectively, and for another proton on a carbon atom carrying an acetoxyl group at δ 5·29 (*d*, *J* 4 Hz). The latter proton, whose location in the D,E ring system was supported by the MS of **1**, can only be accommodated at C-19 since all the remaining possible secondary hydroxy-positions possess at least two neighbouring hydrogens. The coupling constant (4 Hz) suggested that sericic acid is either 18 α-H, 19 β-OH or 18 β-H, 19 α-OH. Inversion at C-18 was achieved by acetylation of **3** under forcing conditions[8] which gave a new tetra-acetate (**12**). To this derivative the 18-*iso* (α-) structure was ascribed since its NMR spectrum exhibited an *ax–ax* coupling (12 Hz) between the protons at C-18 and C-19. Sericic acid is therefore 2α,3β,19α,24-tetrahydroxy-olean-2-en-28-oic acid (**1**).

Sericoside (**13**), IR absorption at 1730 and 3100–3600 cm^{-1}, did not react with diazomethane. After acetylation a hepta-acetate (**14**) was obtained, which showed IR absorption at 3440 cm^{-1} (incomplete acetylation) and seven NMR acetyl signals between δ 1·86–2·06. Acidic hydrolysis of **13** yielded *D*-glucose and sericic acid, while $LiAlH_4$ reduction gave the pentahydroxy derivative **15** which was characterized as the tetra-acetate **16** identical with the same compound prepared from **1**. Exhaustive methylation of **13** and acidic hydrolysis afforded 2,3,4,6-tetra-*O*-methyl-*D*-glucose. On the basis of these data sericoside was assigned structure **13**. The nature of the glycosidic linkage was clearly shown to be β by the signal, in the NMR spectrum of **14**, at δ 5·60 (1H, *d*, *J* 8 Hz) attributable to the axial C-1 proton of the glucose moiety.

EXPERIMENTAL

The plant material was provided by M. F. de Carvalho, Scientific Investigation Inst., Agronomic Sect., Lourenço Marques (Mozambique). A herbarium specimen is available at the Dept of Pharmacognosy, Univ. della Beffa, Milan, Italy. M.ps are corrected. The NMR spectra were recorded at 100 MHz and at 60 MHz.

Isolation of the triterpenoids. The dry powdered roots (1·47 kg) were refluxed in 90% MeOH several times and the extract filtered hot, conc and diluted with H_2O. The dark brown aq. soln was shaken with $CHCl_3$, and then with *n*-BuOH. The $CHCl_3$ extracts were evaporated to dryness and the residue chromatographed on silica gel using EtOAc–MeOH (95:5) as eluent; 780 mg of sericic acid (**1**) were obtained. The BuOH extracts were conen, diluted with Et_2O and the solid material collected and dried. After column chromatography [silica gel, $CHCl_3$–MeOH–H_2O (13:7:2), lower phase], 2·1 g of sericoside (**13**) were obtained.

Sericic acid (**1**) showed the following properties: m.p. 282° (from EtOH), $[\alpha]_D^{30}$ +38° (c 0·32, EtOH). IR (KBr): 3450, 1705 cm^{-1}. MS: *m/e* 504 (M^+, 3%), 486 (2), 468 (2), 442 (6), 264 (95), 246 (65), 240 (9), 231 (52), 219 (17), 201 (100). (Calc. for $C_{30}H_{46}O_6$: C, 71·34; H, 9·60. Found: C, 71·28; H, 9·67.)

Sericoside (**13**) showed the following properties: m.p. 209° (from MeOH), $[\alpha]_D^{30}$ +5·4° (c 2, pyr). IR (KBr): 3600–3100, 1730 cm^{-1}. (Calc. for $C_{36}H_{58}O_{11}$: C, 64·82; H, 8·77. Found: C, 64·73; H, 8·85.)

Sericic acid methyl ester (**2**). Excess CH_2N_2 was added to a MeOH soln of **1** (300 mg); the methyl ester crystallized from MeOH, m.p. 160°, $[\alpha]_D$ +41° (c 0·3, EtOH). M^+ 518. IR (KBr): 3420, 1725 cm^{-1}. (Calc. for $C_{31}H_{50}O_6$: C, 71·76; H, 9·72. Found: C, 71·69; H, 9·70.)

Sericic acid methyl ester triacetate (**3**). 90 mg of **2** in 2 ml of C_5H_5N were treated with Ac_2O (1 ml) for 24 hr at room temp. Dilution with H_2O and crystallization from aq. EtOH gave **3**, m.p. 178°, $[\alpha]_D$ −10° (c 2, pyr). IR ($CHCl_3$): 3640, 1750 cm^{-1}. (Calc. for $C_{37}H_{56}O_9$: C, 68·89; H, 8·75. Found: C, 68·91; H, 8·77).

Sericic acid methyl ester tetra-acetate (**4**). A drop of $HClO_4$ was added at −10° to a soln of **3** (45 mg) in Ac_2O (2 ml) and the mixture was kept at room temp. for 15 min. Dilution with H_2O and crystallization from aq. EtOH yielded **4**, m.p. 223°. IR (KBr): 1740 cm^{-1}. (Calc. for $C_{39}H_{58}O_{10}$: C, 68·17; H, 8·51. Found: C, 68·09; H, 8·58.)

19-*Keto sericic acid methyl ester triacetate* (**5**). A soln of **3** (35 mg) in Me_2CO (3 ml) was treated at 10° with 0·4 ml of Jones reagent for 15 min. Dilution with H_2O and extraction with Et_2O gave pure **5**, m.p. 200° (from aq. MeOH). $[\alpha]_D$ +33° (c 0·5, EtOH). IR (KBr): 1745, 1735, 1710 cm^{-1}. NMR (60 MHz, $CDCl_3$) δ: 5·28 (1H, *m*, C-12 H), 5·03 (1H, *m*, C-2 H), 4·82 (1H, *d*, *J* 10 Hz, C-3 H), 4·18 (2H, *s*, C-24 H_2), 3·72 (1H, *bs*, C-18 H), 3·65 (3H, *s*, $-CO_2Me$), 2·08 (6H, *s*, OCOMe), 2·00 (3H, *s*, $-OCOMe$). (Calc. for $C_{37}H_{54}O_9$: C, 83·08; H, 10·18. Found: C, 82·99; H, 10·10.)

[8] Ruzicka, L., Grob, A., Egli, R. and Jeger, O. (1943) *Helv. Chim. Acta* **26**, 1218.

Sericic acid bromo-γ-lactone (**6**). A soln of **1** (26 mg) in MeOH (2 ml) was treated with Br_2 (10 mg) in MeOH (1 ml). After 30 min the soln was cooled to give crystals of **6**, m.p. 228°, IR (KBr): 1760 cm^{-1}. (Calc. for $C_{30}H_{47}O_6Br$: C, 61·72; H, 8·12; Br, 13·69. Found: C, 61·69; H, 8·15; Br, 13·71.)

Oxidation of **1** *with sodium metaperiodate.* A MeOH soln of **1** (150 mg) was treated at room temp. with aq. $NaIO_4$ (10 ml, 0·1 N). After 30 min white needles of **7** crystallized, m.p. 214°, which were dissolved in MeOH and treated with an excess of CH_2N_2. Evaporation to dryness and crystallization from aq. MeOH gave **8**, m.p. 133°, $[\alpha]_D$ +77° (c 2, pyr); 100 mg of **8** were treated for 16 hr at room temp. with Ac_2O (1·5 ml) in C_5H_5N soln. Usual working up gave **9**, m.p. 119°, $[\alpha]_D$ +75° (c 2, pyr), IR (KBr): 3560, 1730 cm^{-1}. (Calc. for $C_{33}H_{48}O_6$: C, 73·28; H, 8·95. Found: C, 73·19; H, 8·98.)

Sericic acid methyl ester tribenzoate (**10**). Two drops of PhCOCl was added at −5° to a soln of **2** (30 mg) in C_5H_5N. After 24 hr, dilution with H_2O and extraction with Et_2O gave **10**, m.p. 199° (from EtOH). λ_{max}(MeOH) 230 nm (log ε 4·55), IR (KBr): 3530, 1720, 1600, 1585, 710 cm^{-1}. (Calc. for $C_{52}H_{62}O_9$: C, 75·13; H, 7·52. Found: C, 75·17; H, 7·54.)

Asiatic acid methyl ester tribenzoate (**11**). Asiatic acid (200 mg) was dissolved in MeOH and treated with CH_2N_2. After evaporation to dryness, the residue was treated for 16 hr with 5 drops of PhCOCl in C_5H_5N soln. Usual working up gave **11**, m.p. 236° (from EtOH), λ_{max}(MeOH) 230 nm (log ε 4·58), IR (KBr): 1725, 1600, 1584, 710 cm^{-1}. (Calc. for $C_{52}H_{62}O_8$: C, 76·61; H, 7·67. Found: C, 76·65; H, 7·64.)

Sericic acid 18-α-H methyl ester tetra-acetate (**12**). A soln of **3** (90 mg) in HOAc containing 40% HBr was kept at room temp. for 24 hr; dilution with cold H_2O, filtration of the ppt and crystallization from *n*-hexane gave **12** (65 mg), m.p. 195°, $[\alpha]_D$ +13° (c 0·4, EtOH), IR (KBr): 1748, 1725 cm^{-1}. NMR (60 MHz, $CDCl_3$)δ: 5·43 (1H, *m*, C-12 H), 5·22 (1H, *m*, C-2 H), 4·86 (1H, *d*, *J* 12 Hz, C-19 H), 4·80 (1H, *d*, *J* 11 Hz, C-3 H), 4·14 (2H, *s*, C-24 H_2), 3·59 (3H, *s*, $-CO_2\underline{Me}$), 2·86 (1H, *bd*, *J* 12 Hz, C-18 H), 2·11 (6H, *s*, $-OCO\underline{Me}$), 2·06 (6H, *s*, $-OCO\underline{Me}$). (Calc. for $C_{39}H_{58}O_{10}$: C, 68·17; H, 8·51. Found: C, 68·12; H, 8·54.)

Sericoside hepta-acetate (**14**). 100 mg of sericoside were acetylated under standard conditions. **14** showed: m.p. 252° (from *iso*-Pr_2O), $[\alpha]_D$ −9° (c 2, pyr). IR (KBr): 3340, 1740 cm^{-1}, NMR (100 MHz, $CDCl_3$)δ: 5·60 (1H, *d*, *J* 8 Hz), 5·5–4·7 (9H as a complex system), 4·24 (2H, *s*, C-24 H_2), 3·34 (1H, *bd*, *J* 3 Hz, C-18 H), 3·08 (1H, *d*, *J* 3 Hz, C-19 H), 2·06–1·86 (7 $-OCO\underline{Me}$ groups). (Calc. for $C_{57}H_{76}O_{14}$: C, 69·90; H, 7·21. Found: C, 69·81; H, 7·28.)

Acidic hydrolysis of sericoside. A soln of **13** (100 mg) in Me_2CO was treated with 2 drops of conc H_2SO_4; after 24 hr, dilution with H_2O, filtration and crystallization from EtOH gave **1** (40 mg). The mother liquors, examined by PC, contained glucose.

Li AlH_4 reduction of sericoside. Treatment of **13** (100 mg) with $LiAlH_4$ in dry THF under standard conditions yielded **15**, which, after acetylation afforded **16** (25 mg), amorphous solid. IR (KBr): 3560, 1740 cm^{-1}. NMR (60 MHz, $CDCl_3$)δ: 5·45 (1H, *m*, C-12 H), 5·35 (1H, *m*, C-2 H), 4·72 (1H, *d*, *J* 11 Hz, C-3 H), 4·11 (2H, *s*, C-24 H_2), 3·74 (2H, *dd*, *J* 11 Hz, C-28 H_2), 3·21 (1H, *bd*, *J* 3 Hz, C-19 H), 2·73 (1H, *d*, *J* 3 Hz, C-18 H), 2·01 (9H, *s*, $OCO\underline{Me}$), 1·93 (3H, *s*, $OCO\underline{Me}$).

Exhaustive methylation of **13** (NaH, MeI, DMF) and *acid hydrolysis* (dil. H_2SO_4, Me_2CO) yielded, after column chromatography (silica gel, C_6H_6–Me_2CO, 9:1), 2,3,4,6-tetra-*O*-methyl-*D*-glucose.