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AN E-RING γ-LACTONE PENTACYCLIC TRITERPENE FROM *MYRIANTHUS SERRATUS*

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Key Word Index—*Myrianthus serratus*; Cecropiaceae; phytochemistry; pentacyclic triterpenes; γ-lactone; serratolide; tormentic acid.

Abstract—A novel γ-lactone triterpenoid has been isolated from the methylated ethyl acetate extract of the trunk wood of *Myrianthus serratus*, as well as friedelin and four known pentacyclic triterpenoid acids, all as their methyl esters. Their structures were elucidated by 2D NMR experiments. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

In our systematic study of the chemical constituents of the Cameroonian members of the family Cecropiaceae, we investigated the trunk wood of *Myrianthus serratus* Trecul, a minor species of this taxon which grows in littoral zones [1] and for which no phytochemical study has yet been published. Previous studies on this family led to the isolation of many pentacyclic triterpenes among which were showing 2,3 oxidative cleavage and A-ring contraction [2, 3]. The present study deals with the same structure elucidation of a new pentacyclic triterpene (**1**) with a γ-lactone system in ring E, obtained together with five known pentacyclic triterpenes including friedelin, methyl tormentate (**2**), methyl euscaphate (**3**), methyl benthamate (**4**) and methyl 2α-hydroxyoleanolate (**5**), which were characterized by comparison of their spectral data with those of authentic samples. The original spectral data of known compounds are obtainable from the author of correspondence.

RESULTS AND DISCUSSION

The ethyl acetate extract of the defatted trunk wood of *M. serratus* was methylated and afforded after repeated vacuum liquid chromatography (VLC) and crystallization four methyl esters of pentacyclic triterpenoid acids: methyl tormentate (**2**) [4], methyl euscaphate (**3**) [5], methyl benthamate (**4**) [6], methyl 2α-hydroxyoleanolate (**5**) [7] and a new E-ring containing γ-lactone derivative, serratolide (**1**).

Compound **1** gave a positive Liebermann–Burchard test. The EIMS showed the $[M]^+$ at m/z 512 corresponding to the molecular formula $C_{32}H_{48}O_5$. Its IR spectrum exhibited absorption bands at 1390 and 1375 (gem-dimethyl), 1765 (γ-lactone) and 1724 (ester), 3430 (OH) and 1605 cm^{-1} (trisubstituted double bond). Interpretation of the ^1H and ^{13}C NMR spectral data by means of ^1H–^1H-COSY, ^1H–^{13}C COSY and HMBC led to structure **1**. The ^1H NMR spectrum of **1** showed singlet resonances of six tertiary methyl groups at δ 0.72 (Me-29), 0.80 (Me-24), 1.10 (Me-24), 1.18 (Me-25), 1.27 (Me-27) and 1.42 ppm (Me-29) and a secondary methyl signal at δ 1.11 (*d*, J = 7.5 Hz) corresponding to Me-30, as well as an acetyl group resonance at δ 2.07 attributed to 3-OAc. This was confirmed by the H-2 signal which appeared at δ 4.93 as ddd, with two large (J_1 = 14.5 and J_2 = 10.5 Hz) and one small (J = 4.5 Hz) coupling constants. This chemical shift as well as the coupling pattern were almost identical with those of methyl cecropate (**6**) in which H-2 appeared at δ 4.95 [8], and therefore established an identical substitution of ring A in both compounds and consequently proved H-2 to be β-axial. Accordingly, the

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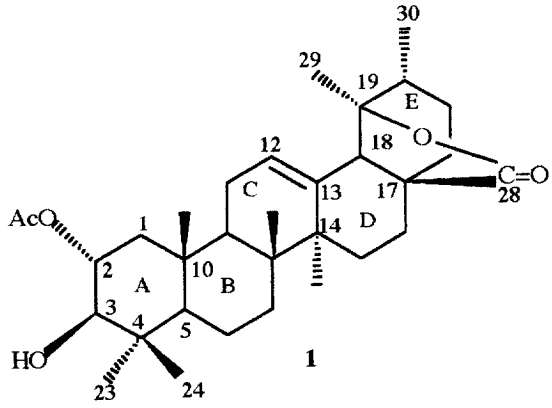

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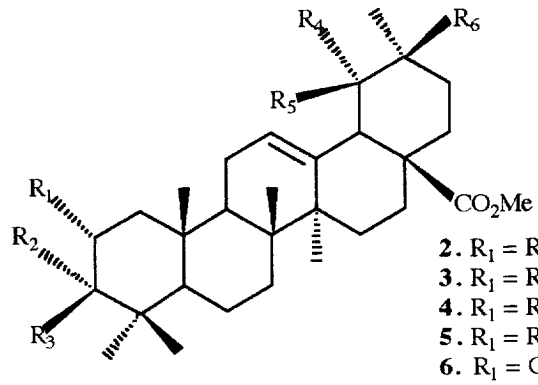

2. $R_1 = R_3 = R_4 = OH$ $R_2 = R_6 = H$ $R_5 = Me$
3. $R_1 = R_2 = R_4 = OH$ $R_3 = R_6 = H$ $R_5 = Me$
4. $R_1 = R_2 = R_6 = H$ $R_3 = R_4 = OH$ $R_6 = Me$
5. $R_1 = R_3 = OH$ $R_2 = R_4 = R_5 = H$ $R_6 = Me$
6. $R_1 = OAc$ $R_2 = R_4 = R_6 = H$ $R_3 = OH$

acetoxyl group at that position was α-equatorial [7]. This was confirmed by the presence of the signal at δ 3.21 which appeared as a doublet (J = 10.5 Hz) attributed to H-3 at an α-axial position geminal to a hydroxyl group. Similarly, H-3 resonates at δ 3.21 in compound **6**. Close inspection of the ^{1}H NMR revealed the presence of the singlet signal at δ 2.52 attributed to H-18, compatible with the absence of a proton at the C-19 position, thus confirming the tertiary nature of that carbon atom. Finally, the vinylic proton at δ 5.32 appeared as a triplet (J = 3.5 Hz) compatible with the existence of the allylic methylene group at C-11. The presence of the secondary methyl group in the ^{1}H and ^{13}C NMR spectra further suggested that compound **1** belonged to the Δ^{12} ursene-type pentacyclic triterpenes. In fact C-12 and C-14 in compound **1** resonated at δ 129.0 and 138.0 in good agreement with urs-12-en derivatives [8]. The downfield effect on Me-29 (d, 1.42 ppm) is rather unusual compared to the chemical shift of that group in other 19α-hydroxylated urs-12-en derivatives [9]. In fact, the Me-29 group in methyl tormentate (**2**) appeared at δ 1.19, where it was geminal to a hydroxyl group. The $\Delta\delta$ = 0.23 suggested the presence of a lactone system between the 19-hydroxyl and 28-carboxyl groups. This was corroborated by the resonances of C-19 and C-28 both in methyl tormentate (19α-hydroxylated derivative) and in compound **1**. The above carbons resonated at δ 73.0 and 178.3, respectively, in methyl tormentate (**2**); however, these carbon atoms appeared at δ 79.1 and 183.7 in compound **1**, thus confirming the presence of the lactone system in ring E. Furthermore, the downfield effect on the Me-29 protons (d, s 1.42) observed on the ^{1}H NMR of compound **1** could be seen as resulting from the magnetic anisotropy generated by the carbonyl function of the lactone system. The configuration of the lactone ring was envisaged to be β, as the result of the epimerization at C-19 concomitant to the change in the junction between rings D and E from *trans* as in methyl tormentate (**2**) to *cis* in serratolide (**1**) (Fig. 1). This was corroborated by molecular model building which showed the inversed boat conformation to be the most plausible for the formation of the lactone ring. As a consequence of the above epimerization, the Me-29 group had to be α. According to the Me-20 group, it has the same orientation as in other similar derivatives (Fig. 1). Structure **1** was further supported by the results from the HMBC experiment, optimized for a coupling constant value of 7 Hz: cross-peaks were observed between H-18 (δ 2.52) and C-19 (δ 79.1), C-17 (δ 47.1) and C-13 (δ 138.0). Cross-peaks were also observed between H-2 and C-3 (δ 80.9) and C-4 (δ 40.0). The EIMS of **1** corroborated

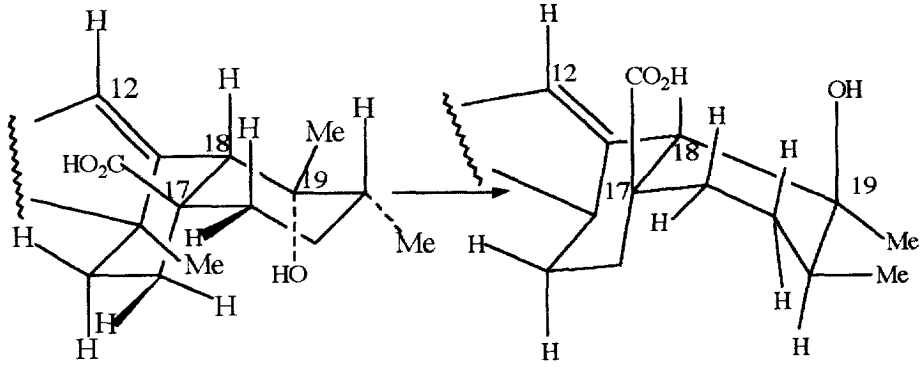

Fig. 1. Perspective drawing indicating the conformation change of ring E leading to γ-lactone formation.

the above structure with important fragments at *m/z* 43 (acetoxyl group), 246 (RDA) and 266 (M − RDA), 485 (M + 1 − CO). This structure was also confirmed by alkaline hydrolysis of **1** which led to tormentic acid (**3**) and the methyl ester of which was identical in all respects (^{1}H, ^{13}C NMR and m.p.) to methyl tormentate (**2**). In the light of the above evidence, compound **1** is 2α-acetoxy,3β-hydroxyurs-12-en-19, 28-olide and has been given the name serratolide.

EXPERIMENTAL

General

^{1}H- and ^{13}C-NMR (1D or 2D experiments): 300 and 75 MHz, respectively, with TMS as int. standard; EIMS: 70 eV; CI-MS: NH_3; IR: KBr; CC: silica gel (70–230 mesh ASTM) (Riedel-de-Haën); TlC: silica gel GF_{254}.

Plant material

The trunk wood of *Myrianthus serratus* Trecul was collected in March 1994 at Dizangue (Cameroon Littoral Province) by Gaston Achoundong of the Cameroon National Herbarium, where a voucher specimen (No. 32440/SRF/CAM) is deposited.

Extraction and isolation of compounds

The plant material was cut into pieces, air-dried and pulverized. The resulting powder (3.5 kg) was continuously extracted by maceration with MeOH (15 l) at room temperature for 3 days and the operation repeated 2 more times. The combined MeOH extracts were concentrated under red. pres. to yield a precipitate which was filtered and dried (38 g). The filtrate was further diluted with H_2O and fractionated successively with hexane and EtOAc. The EtOAc fraction (16 g) had qualitatively the same composition as the above precipitate, and therefore was combined with it and the mixture methylated with an Et_2O solution of CH_2N_2. The solid obtained after evaporation of the solvent (50 g) was repeatedly chromatographed over silica gel (VLC) columns (1.5 kg) eluted with hexane–EtOAc mixtures with increasing polarity. Hexane–EtOAc (9:1) afforded friedelin. Elution with hexane–EtOAc (17:3) gave Me pomolate and 2α-hydroxyoleanolate (**5**), whereas 4:1 mixture furnished Me tormentate (**2**), Me euscaphate (**3**) and the new compound serratolide (**1**).

Table 1. ^{13}C NMR spectral data of serratolide (**1**), methyl tormentate (**2**) and methyl cecropate (**6**)

C	1	2	6
1	46.7	46.3	44.2
2	73.3	68.5	73.5
3	80.9	83.3	81.2
4	40.0	39.1	39.9
5	55.0	55.1	55.4
6	18.3	18.3	18.4
7	37.4	32.4	33.1
8	39.7	39.7	39.8
9	47.7	47.0	47.8
10	38.3	37.9	38.5
11	23.7	23.5	23.6
12	129.0	128.6	125.5
13	138.0	138.0	138.6
14	41.1	41.0	42.3
15	28.1	28.0	28.2
16	24.5	25.2	24.4
17	47.1	47.7	48.3
18	52.8	53.0	53.1
19	79.1	72.8	39.4
20	39.7	41.0	39.1
21	32.5	25.8	30.8
22	37.4	37.2	36.8
23	28.5	28.6	28.7
24	17.0	16.7	17.0
25	16.3	16.4	16.5
26	16.6	16.4	16.7
28	183.7	178.3	178.1
29	16.9	27.2	17.0
30	21.4	15.2	21.1
$COCH_3$	171.4		171.6
	52.8		51.4
OCH_3		51.5	

Saponification of compound **1**

Compound **1** (20 mg) was refluxed overnight in 10% MeOH–KOH (10 ml). The reaction medium was further diluted with H_2O (10 ml) and neutralized with 0.1 N HCl. Extraction of the reaction medium with EtOAc and evaporation to dryness afforded tormentic acid.

2α-Acetoxy,3β-hydroxyurs-12-en-19,28-olide

Amorphous powder (hexane–EtOAc). IR, ν_{max} cm^{-1}: 3430 (*br* OH), 1765 (γ-lactone), 1725 (ester), 1605 (>C=CH–), 1390–1375 (gem-dimethyl), 1200 (C–O), 1030, 920, 860, 795 and 765 cm^{-1}; ^1H NMR: δ 0.72 (3H, *s*, CH_3-26), 0.80 (3H, *s*, CH_3-24), 1.10 (3H, *s*, CH_3-23), 1.11 (3H, *d*, J = 7.5 Hz, CH_3-30), 1.18 (3H, *s*, CH_3-25), 1.27 (3H, *s*, CH_3-27), 1.42 (3H, *s*, CH_3-29), 0.94 (1H, *m*, H-1ax), 1.90 (1H, *m*, H-1eq), 2.00 (1H, *m*, H_2-11), 2.07 (3H, *s*, 2-$COCH_3$), 2.52 (1H, *s*, H-18), 3.21 (1H, *d*, H-3), 4.93 (1H, *ddd*, J_1 = 14.5, J_2 = 10.5, J_3 = 4.5 Hz). ^{13}C NMR: Table 1.

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REFERENCES

1. Berg, C. C, Hijman, M. E. E. and Weerdenburg, J. C. A., *Flore du Cameroun*, Vol. 28, ed. B. Satabié. MESRES, Yaounde, Cameroon, 1985, pp. 262–270.
2. Lontsi, D., Sondengam, B. L., Martin, M. T. and Bodo, B., *Phytochemistry*, 1991, **30,** 1621.
3. Lontsi, D., Sondengam, B. L., Martin, M. T. and Bodo, B., *Phytochemistry*, 1991, **30,** 2361.
4. Ojinnaka, C. M., Okogun, J. I. and Okorie, D. A., *Phytochemistry*, 1980, **19,** 2482.
5. Lontsi, D., Sondengam, B. L., Martin, M. T. and Bodo, B., *Phytochemistry*, 1992, **31,** 4285.
6. Barmejio, J., Breton, J. L., De la Fuente, G. and Gonzales, A. G., *Tetrahedron Letters*, 1967, **47,** 4649.
7. Glen, A. T., Lawrie, W., McLean, J. and Younes, G., *Journal of the Chemical Society*, 1967, 510.
8. Lontsi, D., Sondengam, B. L., Ayafor, J. F., Tsoupras, M. G. and Tabacchi, R., *Planta Medica*, 1990, **56,** 287.
9. Seo, S., Tomita, Y. and Tori, K., *Tetrahedron Letters*, 1975, **1,** 7.