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CECROPIOIC ACID, A PENTACYCLIC TRITERPENE FROM *MUSANGA CECROPIOIDES*

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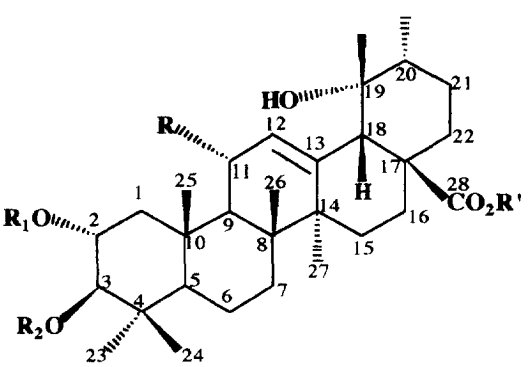
Key Word Index—*Musanga cecropioides*; Cecropiaceae; rootwood, cecropioic acid; pentacyclic triterpenes.

Abstract—Six pentacyclic triterpenes were isolated as their methyl ester from the methylated rootwood extracts of *Musanga cecropioides* and their structure elucidated by 2D-COSY spectroscopic methods. They were methyl tormentate, methyl 2-acetyltormentate, methyl 28-glucosyltormentate, methyl pomolate, methyl euscaphate and a new pentacyclic triterpene, methyl cecropioate (methyl 2α,11α-diacetoxy,3β,19α-dihydroxyurs-12-en-28-oate).

INTRODUCTION

Musanga cecropioides R. Br. ex-Ted [1] is an endemic secondary forest tall tree in the tropical African zone which ranges from Sierra Leone to Angola through Guinea, Uganda and Zaire [2, 3]. The plant has a serious reputation in this zone for the treatment of many diseases, and particularly for use as an anti-hypentensive, anti-diabetic and galactogenic agent and also to facilitate child birth [3–5]. We previously described the structure of triterpenes with oxidative modifications of the A-ring such as ring cleavage and ring contraction [6–10]. The present work deals with the structure of the new pentacyclic triterpene 2α. 11α-diacetoxy-3β,19α-dihydroxyurs-12-en-28-oic acid, isolated as its methyl ester **1**, in addition to the known methyl tormentate (**2**), methyl 2-acetyltormentate (**3**) 28-glucosyl tormentate, and methyl pomolate [11]. A complete ^{1}H NMR and ^{13}C NMR chemical shift assignment of the new compound **1** as well as for the known methyl tormentate (**2**) methyl 2-acetyl tormentate (**3**) and the diacetylated derivative of methyl tormentate (**4**) have been achieved from 2D-NMR experiments and their stereochemistry confirmed.



1 R_1= Ac R_2= H R= AcO R'= Me
2 R_1= H R_2=H R= H R'= Me
3 R_1= Ac R_2=H R= H R'= Me
4 R_1= Ac R_2=Ac R=H R'= Me

RESULTS AND DISCUSSION

Repetitive chromatography of the methylated ethyl acetate extract of air-dried rootwood yielded in addition to known triterpenes such as methyl tormentate (**2**), methyl 2-acetyltormentate (**3**) and glucosyl tormentate (**7**), a small quantity of the new derivative **1**.

Methyl tormentate (**2**), obtained as colourless needles from hexane–ethyl acetate, mp 157–160°C, gave a positive Liebermann-Burchard test, and the EI mass spectrum exhibited a molecular ion at *m/z* 502 in agreement with the molecular formula $C_{31}H_{50}O_5$. Its IR spectrum showed absorption bands at ν_{max} 3540 (alcohol), 1726 (ester), 1655 (trisubstituted double bond) and (1381–1370 cm^{-1}) (gem-dimethyl groups). Compound **2** gave upon acetylation a diacetyl derivative **4** ($C_{35}H_{54}O_7$) which still showed an alcohol function absorption at ν_{max} 3500 cm^{-1}. This suggested the

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structure to be methyl tormentate (**2**) with the presence of alcohol functions among which one was in a hindered position (a tertiary alcohol function).

The ^{13}C NMR spectrum of **2** (Table 1) exhibited carbon atoms including one carbonyl, two sp2 carbon atoms of a carbon-carbon double bond, two methinoxy and one oxygen bearing quaternary carbon. Total assignments of ^1H NMR and ^{13}C NMR spectra were effected on the basis ^1H-^1H COSY, ^1H-^{13}C COSY and long-range ^1H-^{13}C COSY optimized for J_{CH} coupling constants of 7 Hz [12]. From the two former techniques, the different substructures of compound **2** were established, and from the latter technique as well as with INADEQUATE experiments [13], the different substructures were interconnected to form the skeleton of the molecule. From the ^1H NMR spectrum of **2** and subsequent analyses, many structural features could be obtained: two vicinal hydroxy methines at C-2 and C-3 producing signals at δ_H 2.98 and 3.36, respectively, which were further shifted to δ_H 4.72 and 5.07, respectively, in the diacetyl derivative **4**. The proton at C-3 appeared as a doublet ($J = 9.5$ Hz) in good accord with a *trans*-diaxial disposition with H-2. Therefore, H-3 was α-axial oriented and accordingly H-2 was in a β-axial position. This was confirmed by the coupling pattern of H-2 which appeared as a *ddd*, consisting of two large vicinal coupling constants, one with H-3 ($J = 9.5$ Hz), the second with H-1ax ($J = 14.5$ Hz) and a small coupling constant with H-1eq. The configuration of ring A was then determined with the C-2 (OH) in the α-equatorial orientation and the C-3 (OH) on the β-equatorial disposition. The location of the hindered hydroxyl group was deduced from the coupling pattern of H-18 which appeared as a singlet at δ_H 2.57 on the ^1H NMR spectrum of **2**, in good accord with the presence of the hydroxyl at C-19.

Table 1. ^{13}C NMR data of compounds **1**–**4** ($CDCl_3$; TMS as internal standard, 75.47 MHz)

C	1	2	3	4
1	43.58 *t*	46.34 *t*	43.67 *t*	43.87 *t*
2	73.05 *d*	68.47 *d*	73.28 *d*	70.05 *d*
3	80.68 *d*	83.25 *d*	80.87 *d*	80.61 *d*
4	39.75* *s*	39.14 *s*	39.92* *s*	39.31 *s*
5	54.85 *d*	55.05 *d*	55.00 *d*	54.75 *d*
6	18.33 *t*	18.28 *t*	18.36 *t*	18.26 *t*
7	32.92 *t*	32.44 *t*	32.56 *t*	32.48 *t*
8	38.81* *s*	39.72 *s*	39.72* *s*	39.90 *s*
9	49.74 *d*	46.95 *d*	47.10 *d*	47.06 *d*
10	44.43 *s*	37.94 *s*	38.19 *s*	38.02 *s*
11	80.99 *t*	23.54 *t*	23.73 *t*	23.70 *t*
12	128.25 *d*	128.59 *d*	128.79 *d*	128.64 *d*
13	144.97 *s*	137.99 *s*	138.14 *s*	138.18 *s*
14	41.59 *s*	40.99 *s*	41.15 *s*	41.14 *s*
15	28.50 *t*	27.99 *t*	28.11 *t*	28.10 *t*
16	25.24 *t*	25.23 *t*	25.41 *t*	25.38 *t*
17	47.49 *s*	47.68 *s*	47.82 *s*	47.81 *s*
18	52.70 *d*	53.02 *d*	53.14 *d*	53.13 *d*
19	73.09 *s*	72.83 *s*	73.13 *s*	73.10 *s*
20	41.05 *d*	40.99 *d*	41.06 *d*	41.07 *d*
21	25.86 *t*	25.83 *t*	25.96 *t*	25.96 *t*
22	37.22 *d*	37.24 *d*	37.33 *d*	37.33 *d*
23	28.51 *q*	28.55 *q*	28.49 *q*	28.38* *q*
24	16.53 *q*	16.72 *q*	16.61** *q*	17.60* *q*
25	17.62 *q*	16.36 *q*	16.23** *q*	16.30* *q*
26	18.35 *q*	16.42 *q*	16.58** *q*	16.54* *q*
27	22.97 *q*	24.37 *q*	24.49 *q*	24.41 *q*
28	178.13 *s*	178.26 *s*	178.28 *s*	178.30 *s*
29	27.46 *q*	27.18 *q*	27.36 *q*	27.37 *q*
30	16.07 *q*	15.97 *q*	16.10 *q*	16.10 *q*
OMe	51.73 *q*	51.46 *q*	51.60 *q*	51.60 *q*
OAc(CO)	172.10 *s*	—	171.61 *s*	170.87 *s*
	171.03 *s*	—	—	176.66 *s*
OAc(Me)	21.50 *q*	—	21.36 *q*	21.13 *q*
	21.05 *q*	—	—	20.91 *q*

* May be reversed within the same column.

The configuration of the compound **2** was further confirmed by the NOE difference measurements [13, 14]. Thus the 25-CH_3 (in β-position) showed a NOE effect (1.5%) with H-2. However, no NOE effect was observed between H-2 and H-3 which have been shown to be in a *trans*-diaxial disposition, but observed between H-3 and 23-CH_3 (1.5%), which is compatible with their α-orientation. Important NOE effects were also observed between H_2-11 and 25-CH_3 (5.5%) and 26-CH_3 (3.0%) which are in a β-orientation. Finally, important NOE effects were exhibited between H-18 and H-12 (9.5%), H-20ax (5.3%), H-22ax (4.6%) and 29-CH_3 (4.8%) which in turn showed a NOE effect with H-12 (3.0%). The resulting structure and stereochemistry agreed with that proposed previously [15].

Methyl cecropioate (**1**) was obtained as an amorphous solid. Its IR spectrum showed absorption bands at ν_{max} 3330 (alcohol), 1725 (ester) and 1620 (trisubstituted double bond). Its EI mass spectrum showed M^+ at *m/z* 602 corresponding to the molecular formula $C_{35}H_{54}O_8$ (further confirmed by the ^{13}C NMR and the *J*-modulated spectral data) [14] suggested a triterpene bearing two acetoxyls, two hydroxyls, one carbomethoxyl and a Δ^{12} double bond. In addition, the retro-Diels–Alder cleavage of **1** indicated the location of one acetoxyl and one hydroxy group in rings A/B and another acetoxyl, the carbomethoxyl and the second hydroxyl in rings C/D/E. Furthermore, many features in its EI mass spectrum and its ^1H NMR spectrum indicated that **1** was closely related in structure to methyl tormentate (**2**) or more specifically to methyl 2-acetyl tormentate (**3**).

The ^1H NMR spectrum of **1** clearly showed, in addition to the groups characteristic of a pentacyclic triterpene of ursane type, the methyl signals for two acetyl groups at δ_H 2.07 and 2.02 and one methoxyl group at δ_H 3.58 (Table 2).

The ^{13}C NMR spectrum of **1** confirmed the presence of 35 carbon atoms as well as that of acetyl groups at δ_{CO} 172.10 and 171.03 and δ_{CH_3} 21.30 and 21.05. Total assignments of the ^1H and ^{13}C NMR data (Tables 1

Table 2. ^{1}H NMR data of compounds **1**–**4** ($CDCl_3$, TMS as internal standard, 300.13 MHz)

C	1	2	3	4
1ax	1.20 *m*	0.92 *m*	1.00 *m*	—
eq	2.281 *dd* 13.1,4.4	1.96 *m*	1.99 *m*	—
2ax	4.919 *ddd* 11.5,10.1,4.4	3.363 *ddd* 14.7,9.5,4.7	4.925 *ddd* 14.6,10.0,4.5	5.070 *ddd* 11.4,10.3,3.6
3ax	3.177 *d* 10.1	2.982 *d* 9.5	3.177 *d* 10.0	4.724 *d* 10.3
5	0.85 *m*	0.81 *m*	—	—
6ax	—	1.32 *m*	—	—
	—	1.52 *m*	—	—
7ax	—	1.50* *m*	—	—
eq	—	1.26* *m*	—	—
9	1.817 *d* 8.7	1.65 *m*	1.66 *m*	—
11ax	4.512 *dd* 8.7,3.9	1.98 *m*	1.95 *m*	—
eq	—	1.98 *m*	1.95 *m*	—
12	5.661 *d* 3.9	5.330 *dd* 3.6,3.6	5.320 *dd* 3.5,3.5	5.311 *dd* 3.6,3.6
15ax	—	1.60 *m*	1.57 *m*	—
eq	—	1.00 *m*	1.02 *m*	—
16ax	2.525 *ddd* 14.5,11.5,4.7	2.489 *ddd* 14.5,12.6,4.6	2.481 *dd* 14.4,12.6,4.8	2.484 *ddd* 14.0,11.0,4.5
eq	—	1.56 *m*	1.57 *m*	—
18	2.658 *s*	2.569 *s*	2.570 *s*	2.575 *s*
20	—	1.38 *m*	1.40 *m*	—
21ax	—	1.25 *m*	—	—
eq	—	1.66 *m*	—	—
22ax	—	1.52 *m*	—	—
eq	—	1.72 *m*	—	—
23	1.036* *s*	1.005 *s*	1.034* *s*	0.881* *s*
24	0.851* *s*	0.801 *s*	0.845 *s*	0.871* *s*
25	1.103 *s*	0.952 *s*	1.004* *s*	1.123* *s*
26	0.696 *s*	0.651 *s*	0.657 *s*	0.641 *s*
27	1.316 *s*	1.229 *s*	1.228 *s*	1.217 *s*
29	1.316 *s*	1.185 *s*	1.174 *s*	1.173 *s*
30	0.932 *d* 6.6	0.917 *d* 6.7	0.916 *d* 6.7	0.911 *d* 6.6
OMe	3.586 *s*	3.575 *s*	3.579 *s*	3.572 *s*
OAc	2.072 *s*	—	2.047 *s*	2.028 *s*
OAc	2.024 *s*	—	—	1.948 *s*
OH	7.552 *s*	—	—	—

and 2) of compounds **1**, **2** and **3** were obtained from ^{1}H-^{1}H 2D-COSY, ^{1}H-^{1}H long range 2D-COSY and ^{1}H-^{13}C 2D COSY. The ^{13}C NMR spectrum of **1** showed the C-2 signal at δ_C 73.05 which is very close to the respective value for methyl 2-acetyl tormentate (**3**), but very different from that of methyl tormentate (**2**) which appeared at δ_C 68.47. Also, the C-3 carbon atoms of compounds **1** and **3** were observed at very close frequencies at δ_C 80.68 and 80.87 respectively. All these facts established the identity of ring A substitution in **1** and **3**. However, the C-3 carbon atom in methyl tormentate **2** resonated at δ_C 83.25. The upfield effect in **1** and **3** with respect to **2** could be interpreted as the β-effect on C-3 due to the presence of the acetoxy group on C-2. Furthermore comparison of the ^{13}C NMR spectral data of **1** and **3** exhibited almost the same chemical shifts for the rest of the carbons except that of C-11, which resonated at δ_C 80.99 instead of δ_C 23.73 as in compounds **2** and **3**. This was consistent with the location of the second acetoxy group at C-11. The stereochemistry of the first acetoxy group on C-2 in **1** was exactly the same as that in compound **3**. However, that of the substituent at C-11 remained to be determined. In fact, the methinoxy proton H-11 resonated at δ 4.51 as a doublet of doublets with a larger and a smaller coupling constants. The larger one (J = 8.7 Hz) could be seen as the result of a *trans*-diaxial coupling with the α-axial proton at C-9 (δ_H 1.87) and the smaller one (J = 3.9 Hz) as the interaction of the same proton with the vinylic hydrogen atom at C-12. Accordingly, the methinoxy proton H-11 is β-axial and therefore the acetoxy group at that position is α-equatorial. The presence of the C-11-acetoxy group was further corroborated by the downfield effect of H-12 which appeared at δ_H 5.66 (*d*, J = 3.9 Hz) instead of around δ_H 5.33 as in methyl tormentate **2** or in methyl 2-acetyl tormentate **3** (*dd*, J = 3.5, 3.5 Hz).

The configuration of the 11-acetoxy group was further confirmed by the observation of NOE difference effects. Thus, an important NOE effect was observed between H-11 and the 25-CH_3 group, which is known

to be β-oriented. This confirmed the orientation of H-11 in the β-position and therefore that of the acetoxyl group at that position as α-located. Apart from the case of H-11, all the NOE effects observed for the different spin systems in methyl tormentate (**2**) were also observed in the case of compound **1**. The different spectral analyses were thus in accord with structure **1** leading to 2α,11α-diacetoxy-3β,19α-dihydroxyurs-12-en-28-oic acid for the natural compound which was termed cecropioic acid.

EXPERIMENTAL

General

IR: KBr discs, ^{1}H and ^{13}C-NMR 300 MHz (^{1}H) and 75 MHz (^{13}C). Chemical shifts of coupled protons were measured either from 1D or from 2D COSY spectra for complex spin systems, using TMS as internal standard. EIMS: Nermag Sidar V 3.0 mass spectrometer. TLC: precoated silica gel plats F_{254} (Merck).

Plant material

The rootwood of *Musanga cecropioides* R. Brown was collected at Nkoa-Mbang, Yaoundé zone, Cameroon, in May 1989. A voucher specimen is deposited at the National Herbarium (Yaoundé, Cameroon).

Extraction and isolation

The rootwood of the plant was cut into pieces, air-dried and pulverized to give a powder (3.8 kg) which was macerated at room temp with MeOH for 3 days. The MeOH extracts were combined and concentrated to dryness to give a solid which was treated as in Ref. [10] to yield an *n*-BuOH extract. The latter in turn was methylated with an excess of an ethereal soln of CH_2N_2. Chromatography of the resulting mixture on silica gel and elution with CH_2Cl_2–AcOEt (4:1) and with hexane–EtOAc (7:3) afforded **1**, **2** and **3**. During concentration of the above mentioned MeOH soln, a ppt was obtained which on purification furnished compound **5** crystallized as colourless needles in EtOAc–MeOH (95:5).

Methyl 2α,11α-diacetoxy-3β,19α-dihydroxyurs-12-en-28-oate (methyl cecropioate) (**1**). $C_{35}H_{54}O_8$. Amorphous solid. EIMS, *m/z* (rel. int.): 602 $[M]^+$, 559 $([M-Ac]^+$ (10), 558 (9), 542 $[M-AcOH]^+$ (15), 526 (5), 512 (7), 499 (37), 483 (56), 465 (11), 429 (12), 423 (14), 383 (14), 355 (13), 333 (29), 293 (48), 281 (12), 255 (28), 221 (26), 205 (25), 201 (26), 187 (32), 185 (28), 161 (24), 147 (38), 133 (41), 119 (100), 107 (40), 105 (40), 99 (31), 95 (34), 83 (20), 69 (24), 55 (50).

Methyl tormentate (**2**). $C_{31}H_{50}O_5$; crystals, m.p. 157–160°C (hexane–EtOAc); IR ν_{Max}^{KBr} cm^{-1}: 3540 (OH), 2934 (C—H), 1720 (ester), 1655 (C—CH=C<), 1460, 1381–1370, 1268, 1200, 1158, 1042, 940, 860, 820, 760, 680. EIMS: H^+ 502. ^{1}H NMR (300 MHz, $CDCl_3$): Table 1. ^{13}C NMR (75 MHz, $CDCL_3$), Table 2.

Methyl 2-acetyltormentate (**3**). $C_{33}H_{52}O_6$, amorphous, ^{1}H NMR (300 MHz, $CDCl_3$): Table 1. ^{13}C NMR (75 MHz, $CDCl_3$): Table 2.

Acetylation of methyl tormentate (**3**). Methyl tormentate (0.3 g) was treated with a mixture of Ac_2O–pyridine (5 ml each) at room temp. overnight. The reaction medium was evad to dryness to yield the diacetate **4** as an amorphous solid. $C_{35}H_{54}O_7$. IR: ν_{Max}^{KBr} (cm^{-1}): 3500 (OH), 2946, 2878, 1742, 1664, 1614, 1550, 1460, 1372, 1252, 1160, 1030, 958, 876. ^{1}H NMR (300 MHz, $CDCl_3$): Table 1. ^{13}C NMR (75 MHz, $CDCl_3$): Table 2. CIMS (NH_3) *m/z* 604 $[M+NH_4]^+$, 587 $[M+H]^+$, 527 $[MH-AcOH]^+$.

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