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3β-HYDROXY-21β-*E*-CINNAMOYLOXYOLEAN-12-EN-28-OIC ACID, A TRITERPENOID FROM *ENTEROLOBIUM CONTORSTISILIQUUM**

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Key Word Index—*Enterolobium contorstisiliquum*, Leguminosae, Mimosoideae, triterpenes, 3β-hydroxy-21β-*E*-cinnamoyloxyolean-12-en-28-oic acid, 3β,21β-dihydroxyolean-12-en-28-oic acid, 21β → 28-lactone, methyl and ethyl esters

Abstract—The triterpenes 3β-hydroxy-21β-*E*-cinnamoyloxyolean-12-en-20-oic acid, 3β,21β-dihydroxyolean-12-en-28-oic acid (machaerinic acid) and its lactone (3β-hydroxyolean-12-en-21β → 28-lactone) were isolated from the fruits of *Enterolobium contorstisiliquum* Methyl and ethyl esters of 3β,21β-dihydroxyolean-12-en-oic acid were isolated and characterized as artifacts The structures of these triterpenes have been established by a study of their chemical and spectroscopic (IR, MS and NMR) data

INTRODUCTION

Enterolobium contorstisiliquum Morang (E Timbouva Mart, *Mimosa contortiliquum* Vell) is an arboreous species which occurs abundantly in the Northeastern Brazilian region and the fruits are known as being toxic to cattle [1], which led to a first chemical investigation [2, 3] We have re-examined the chemical composition of the plant Acid hydrolysis of the ethanolic extract from the fruits afforded the triterpenes **1a** and **2a**, previously isolated [3–5], together with a new compound (**3a**) Methyl (**1b**) and ethyl (**1c**) esters of **1a** were isolated and characterized as artifacts Nomenclature of the lactone follows the rules outlined in a recent review [6]

RESULTS AND DISCUSSION

Hydrolysis of **3a** with 2 N hydrochloric acid (water–methanol) gave **1b**, identical with an authentic sample by mmp, IR and TLC The IR spectrum of compound **3a** exhibited absorption bands at 3350 (OH), 1710 (ester), 1700 (COOH), 1650 (CH=CH), 1600 and 1500 cm^{-1} (arom) The ^{1}H NMR spectrum revealed signals at δ6 40 (*d*, J = 16 Hz, H-8′), 7 62 (*d*, J = 16 Hz, H-7′) and 7 3–7 6 (*m*, 5H arom) which characterized the *E*-cinnamoyloxy group The presence of this group was confirmed by the ^{13}C NMR spectrum [δ166 3 (*s*, C-9′), 118 4 (*d*, C-8′), 144 3 (*d*, C-7′), 134 3 (*s*, C-1′), 127 9 (*d*, C-2′ and C-6′), 128 7 (*d*, C-3′ and C-5′), 130 0 (*d*, C-4′)], aided by a comparative analysis with *E*-cinnamic acid [δ172 2 (COOH), 117 4 (Cα), 146 8 (Cβ), 134 0 (C-1), 128 2 (C-2 and C-6), 128 8 (C-3 and C-5), 130 5 (C-4)] [7]

The EIMS showed a peak at *m/z* 454 ($C_{30}H_{46}O_3$), generated from the molecular ion [$C_{39}H_{54}O_5$, M$^+$ 602 (absent)] through a McLafferty rearrangement with loss of a cinnamic acid molecule ($C_9H_8O_2$) The retro-Diels–Alder fragmentation of the radical ion *m/z* 454, involving the double bond at the C-12 position [8, 9], gave rise to the peak of *m/z* 246 (99%, **6**), which suggested the presence of the *E*-cinnamoyloxy group at C-21

The presence of the double bond at C-12 was confirmed in the ^{13}C NMR spectrum (Table 1) by the chemical shifts of C-12 (δ122 6, *d*) and C-13 (δ142 8, *s*), characteristic of a Δ^{12}-oleonene [10, 11] Additional analysis of the proton coupled (SFORD) and decoupled ^{13}C NMR spectra confirmed the thirty carbons, with the presence of an *E*-cinnamoyloxy group ($C_9H_7O_2$), and established the molecular formula of the basic skeleton ($C_{30}H_{47}O_3$) Two sp^3 carbons bonded to oxygen are represented by doublet signals at δ78 5 and 75 7 One equatorial secondary hydroxyl was assigned to C-3 for biogenetic and spectroscopic reasons ^{1}H NMR (δ3 20, *dd*) and ^{13}C NMR [δ78 5 (C-3), 27 7 (C-2), 38 8 (C-4), 28 1 (C-23), 15 7 (C-24)] The assignment of an equatorial-position for the *E*-cinnamoyloxy group at C-21 was deduced by chemical shifts of the carbons 20, 21, 22, 29 and 30 (δ35 3, 75 6, 36 8, 28 8 and 18 4, respectively) and by the appearance of a signal at δ4 91 (H-3) as a double doublet (J_{aa} = 11, J_{ae} = 7 Hz) This analysis was aided by the comparison with data from model compounds (Table 1) Considering that the chemical shift of C-3 in the ^{13}C NMR of **3a** is similar to that of the methyl oleonolate (**4a**) and different from that of the methyl 3-*O*-acetyloleonolate (**4b**), it would seem that the presence of an *E*-cinnamyloxy group at C-3 must be excluded (Table 1)

Treatment of **3a** with pyridine–acetic anhydride gave the monoacetate derivative **3b** [δ4 52 (*dd*, J = 11, 7 Hz, H-3), 2 05 (*s*, Ac)] The chemical shift and coupling constants (100 MHz, $CDCl_3$, TMS) of the H-3 (δ3 21, *dd*, J = 9 and 7 Hz) of machaerinic acid lactone (**2a**) suggested a 3β-hydroxy group stereochemistry This deduction was confirmed by the chemical shifts of carbons 1, 2,

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3, 4, 5, 23 and 24 (Table 1) The presence of 3α-(**7a**) or 3β-hydroxy group (**7b**) is indicated by the chemical shifts of those carbons [13] These, and other data (Table 1) confirm the proposals of Tursch *et al* [5]

The ^{1}H NMR spectra (100 MHz, $CDCl_3$, TMS) of methyl (**1b**) and ethyl machaerinate (**1c**) were also utilized to define equatorial positions to the hydroxyl groups localized at C-3 and C-21 [**1b** 3 21 (*dd*, *J* = 10 and 7 Hz, H-3), 3 52 (*dd*, *J* = 11 and 5 Hz, H-21), **1c** 3 22 (*dd*, *J* = 10 and 7 Hz, H-3), 3 53 (*dd*, *J* = 11 and 5 5 Hz, H-21)]

Finally, our attention was directed to the presence of a peak at *m*/*z* 207 registered in the mass spectrum of the pentacyclic triterpenes **1a**–**1c**, **2a** and **3a**, with relative intensity 31, 22, 25, 23 and 21 %, respectively This peak probably represents the fragment **8**, formed after an hydrogen rearrangement This proposition is supported by the identical structural system involving the rings A and B of these compounds and by the presence of a peak at *m*/*z* 189 (*m*/*z* 207 – H_2O) The assignment of an ion-radical with *m*/*z* 208, produced by retro-Diels–Alder fragmentation [8], as the principal precursor of the cation **8** must be excluded by the small relative intensity of the *m*/*z* 193 peak (*m*/*z* 208 – Me)

Table 1 ^{13}C NMR chemical shift data of methyl oleanolate (**4a**) and its acetate (**4b**) [10], arjunic acid (**5**) [12], machaerinic acid lactone (**2a**) and 3β-hydroxy-21β-*E*-cinnamoyloxyolean-12-en-28-oic acid (**3a**)

Carbon	(**4a**)	(**4b**)	(**5**)†	(**2a**)	(**3a**)
1	38 5	38 1	47 2	38 1	38 5
2	27 1	23 6	67 1	27 0	27 2
3	78 7	80 7	82 2	78 7	78 5
4	38 7	37 5	38 8	38 7	38 8
5	55 2	55 2	54 8	55 4	55 2
6	18 3	18 2	16 9	18 4	18 4
7	32 6	32 6	32 4	32 4	32 8
8	39 3	39 3	38 9	39 8	39 2
9	47 6	47 5	47 2	47 4	47 6
10	37 0	36 9	38 5	37 3	37 0
11	23 1	23 0	23 2	23 6	23 4
12	122 1	122 1	122 6	121 9	122 6
13	143 4	143 6	143 4	140 3	142 8
14	41 6	41 6	41 9	41 9	41 7
15	27 7	27 7	28 4	27 5	27 8
16	23 4	23 6	24 0	23 8	24 4
17	46 6	46 6	46 6	43 3	47 9
18	41 3	41 1	43 1	39 8	40 7
19	45 8	45 8	80 0	47 4	46 6
20	30 6	30 6	36 9	39 5	35 3
21	33 8	33 3	28 4	84 1	75 6
22	32 3	32 3	34 8	36 7	36 8
23	28 1	28 0	27 1	28 0	28 1
24	15 6*	16 8	16 8	15 4	15 7*
25	15 3*	15 3	16 1	15 4	15 3*
26	16 8	16 8	16 9	16 1	16 9
27	26 0	25 8	28 7	26 1	25 7
28	177 9	177 8	179 0	181 8	178 7
29	33 1	33 1	28 7	27 5	28 8
30	23 6	23 6	24 4	23 6	18 4

*Assignments may have to be reversed

†Using DMSO-d_6–$CDCl_3$ The spectra of **2a** and **3a** were obtained at 25 2 MHz in the Fourier transform mode in $CDCl_3$ solns The δ values are in ppm downfield from TMS

EXPERIMENTAL

Mps were determined on a Kofler hot-stage microscope and are uncorr

Isolation of the constituents of Enterolobium contortsiliquum Fruits of a specimen, (identified by botanist Maria de F Agra, Universidade Federal da Paraiba, João Pessoa), were collected at João Pessoa, Paraiba state After drying, the fruits were reduced to powder (900 g) which was extracted with EtOH The extract (20 g) was dissolved in H_2O and extracted with $CHCl_3$ The aq soln was distilled and the residue was refluxed in 1 N HCl and MeOH for 4 hr This soln was extracted with $CHCl_3$ and the $CHCl_3$ evaporated The residue (10 g) was chromatographed on silica gel (300 g) $CHCl_3$ and mixtures of $CHCl_3$–MeOH of gradually increasing polarities were utilized as eluents The $CHCl_3$ fractions were purified by TLC (silica gel) with $CHCl_3$–MeOH (95 5), furnishing **1b** (80 mg) and **2a** (70 mg) TLC (silica gel) with $CHCl_3$–Me_2CO (7 3) of the $CHCl_3$–MeOH fractions permitted the separation of **3a** (130 mg) and **1a** (50 mg) Repetition of the acid hydrolysis with EtOH gave the ethyl ester (**1c**), besides those of **2a**, **3a** and **1a**, permitting the methyl ester (**1b**) to also be considered as a possible artifact The possibility of transesterification (COOMe → COOEt) cannot be definitively precluded

3β,21β-Dihydroxyolean-12-en-28-oic acid (*machaerinic acid*, **1a**) Mp 290–295° (EtOH) {lit [5] 295–305° ($CHCl_3$–EtOH)} Diacetate (**1d**), mp 266–270° (MeOH) (lit [4] 252–259°)

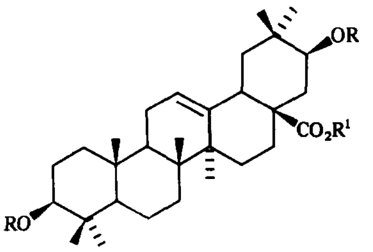
Methyl 3β,21β-dihydroxyolean-12-en-28-oate (*methyl machaerinate*, **1b**) Mp 234–236° (MeOH) {lit [5] 230–233° ($CHCl_3$–MeOH)} Diacetate (**1e**), mp 275–277° (MeOH) {lit [5] 278° ($CHCl_3$–MeOH)}

Ethyl 3β,21β-dihydroxyolean-12-en-28-oate (*ethyl machaerinate*, **1c**) Mp 245–247° (MeOH) MS *m*/*z* (rel int) 500 $[M]^+$ (15), 482 (19), 426 (16), 409 (12), 408 (8), 292 (4), 274 (35), 273 (9), 262 (17), 261 (61), 207 (25), 201 (100), 191 (10), 190 (15), 189 (15), 188 (9), 187 (33) IR ν_{max}^{KBr} cm^{-1} 3400, 1735, 1240 ^{1}H NMR (100 MHz, $CDCl_3$) δ5 32 (*m*, H-12), 4 10 (*q*, *J* = 6 5 Hz, $OC\underline{H}_2$–CH_3), 3 54 (*dd*, *J* = 11 and 6 Hz, H_{ax} – 21), 3 21 (*dd*, *J* = 12 and 6 5 Hz, H_{ax}-3), 1 24 (*t*, *J* = 6 5 Hz, OCH_2–$C\underline{H}_3$), 1 12 (*s*, Me), 1 00 (*s*, 2 × Me), 0 92 (*s*, 2 × Me), 0 78 (*s*, Me), 0 74 (*s*, Me)

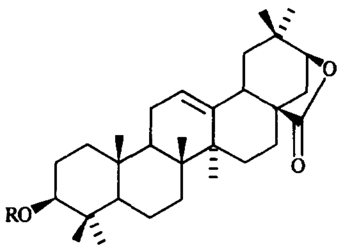
3β-hydroxyolean-12-en-21β → 28-lactone (*machaerinic acid lactone*, **2a**) Mp 260–262° (Me_2CO) {lit [5] 240–243° ($CHCl_3$–MeOH)} Monoacetate (**2b**), mp > 300° (MeOH) (lit [3] 305°)

3β-Hydroxy-21β-cinnamoyloxyolean-12-en-28-oic acid (**3a**) Mp 270° (Me_2CO) MS *m*/*z* (rel int) 602 $[M]^+$ (absent), 454 (15) [found 454 3432, $C_{30}H_{46}O_3$ requires 454 3435], 309 (9), 247 (17), 246 (99), 216 (20), 207 (21), 203 (32), 202 (15), 201 (96), 196 (17), 191 (18), 190 (17), 189 (18), 175 (14), 148 (11), 131 (100) IR ν_{max}^{KBr} cm^{-1} 3350, 1710, 1700, 1640, 1580, 1450, 1380, 1240, 1020, 760 ^{1}H NMR (100 MHz, $CDCl_3$) δ7 3–7 6 (*m*, 5H arom), 7 62 (*d*, *J* = 16 Hz, H-7′), 6 40 (*d*, *J* = 16 Hz, H-8′), 5 34 (*m*, H-12), 4 93 (*dd*, *J* = 11 and 7 Hz, H_{ax}-21), 3 20 (*dd*, *J* = 12 and 7 Hz, H_{ax}-3), 1 14 (*s*, Me), 1 08 (*s*, Me), 0 98 (*s*, Me), 0 92 (*s*, 2 × Me), 0 78 (*s*, 2 × Me) Monoacetate (**3b**), mp 264–265° ($CHCl_3$–MeOH) IR ν_{max}^{KBr} cm^{-1} 1730, 1710, 1690, 1645, 1580, 1460, 1380, 1250, 760 ^{1}H NMR (100 MHz, $CDCl_3$) δ7 3–7 6 (*m*, 5H, arom), 7 66 (*d*, *J* = 16 Hz, H-7′), 6 40 (*d*, *J* = 16 Hz, H-8′), 5 32 (*m*, H-12), 4 94 (*dd*, *J* = 10 and 6 Hz, H_{ax}-21), 4 52 (*dd*, *J* = 11 and 7 Hz, H_{ax}-3), 2 05 (*s*, OAc), 1 15 (*s*, Me), 1 10 (*s*, Me), 0 90 (*s*, 2 × Me), 0 84 (*s*, 2 × Me), 0 76 (*s*, Me)

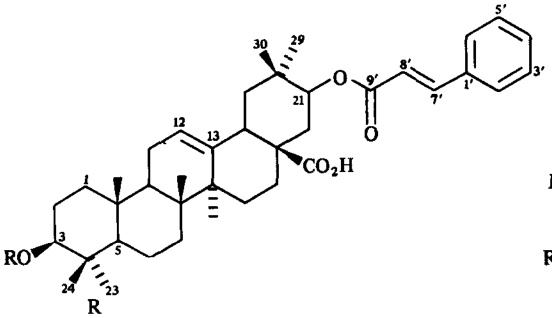
Acid hydrolysis of **3a** *to give* **1b** Compound **3a** was hydrolysed with 2 N HCl (H_2O–MeOH) at room temp (37°) for 17 hr to



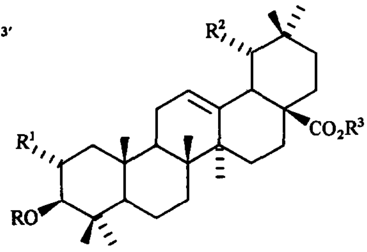
1a $R = R^1 = H$
1b $R = H, R^1 = Me$
1c $R = H, R^1 = Et$
1d $R = Ac, R^1 = H$
1e $R = Ac, R^1 = Me$



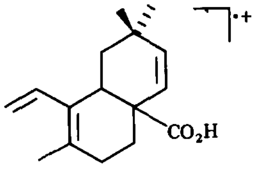
R
2a H
2b Ac



R
3a H
3b Ac



4a $R = R^1 = R^2 = H, R^3 = Me$
4b $R = Ac, R^1 = R^2 = H, R^3 = Me$
5 $R = R^3 = H, R^1 = R^2 = OH$



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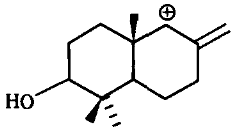

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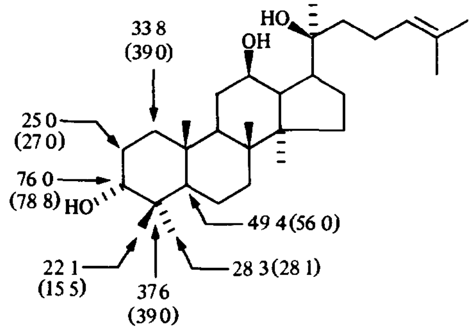

7a $3\alpha-OH$
7b $3\beta-OH$ (δ values in parentheses)

yield methyl machaerinate (**1b**), separated from the mixture on a silica gel column (fraction 3) and crystallized (MeOH) It was identified with authentic sample by mmp, IR and TLC

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