Phytochemistry, Vol 23, No 11, pp 2593–2595, 1984
Printed in Great Britain

0031–9422/84 $3 00 + 0 00

TRITERPENOID ACIDS FROM *SAPIUM SEBIFERUM**

BHIM P PRADHAN†, SIBABRATA DE, AMER NATH and JAMES N SHOOLERY‡

Department of Chemistry, University of North Bengal, P O North Bengal University, Darjeeling, India, ‡NMR Application Laboratory, Varian Associates, Palo Alto, CA 94303, U S A

(*Revised received* 29 *March* 1984)

Key Word Index—*Sapium sebiferum*, Euphorbiaceae, triterpene acids, sebiferic acid, aleuritolic acid, 2α,3β-dihydroxytaraxer-14-en-28-oic acid

Abstract—Sebiferenic acid, isolated from the bark of *Sapium sebiferum*, has been characterized as 2α,3β-dihydroxytaraxer-14-en-28-oic acid on the basis of ^{1}H NMR, ^{13}C NMR and mass spectral evidence and chemical transformations The structure of sebiferic acid has been revised

INTRODUCTION

In our previous communication [1] we reported the isolation of sebiferic acid from the bark of *Sapium sebiferum* Reinvestigation of the acidic portion of the extract from the same plant resulted the isolation of a new pentacyclic triterpenoid acid, sebiferenic acid, besides aleuritolic acid [2] and the previously reported sebiferic acid

RESULTS AND DISCUSSION

The acidic fraction of the benzene extract on chromatography over alumina yielded from the less polar eluate sebiferic acid, the structure of which was earlier wrongly represented as **1a** [1] According to the work of Nakanishi *et al* [3, 4] it is now represented as **1b** as per their revised structure of isohopene [5, 6] The more polar eluant furnished the amorphous solid **2**, the methyl ester of which was identical with methyl aleuritolate **2a** [2] The most polar eluant afforded a new acid, sebiferenic acid **3**, $C_{30}H_{48}O_4$, mp 325°, $[\alpha]_D^{CHCl_3}$ +32°, ν_{max}^{nujol} cm^{-1} 3300–3420 (OH), 1700 (COOH), 820 (>C=C<$_H$) Compound **3** on esterification with diazomethane gave methyl sebiferenate **3a**, $C_{31}H_{50}O_4$, 487 $[MH]^+$, mp 253–254°, $[\alpha]_D^{CHCl_3}$ +15°, ν_{max}^{nujol} cm^{-1} 3350–3420 (OH), 1730 (COOMe), 820 Compound **3a** on acetylation furnished acetyl methylsebiferenate **3b**, $C_{35}H_{54}O_6$, 571 $[MH]^+$, mp 224–226°, $[\alpha]_D^{CHCl_3}$ +12°, ν_{max}^{nujol} cm^{-1} 1725–1735 (OAc and COOMe), 1250, 1230 (2 × OAc), 820 Hydrolysis of **3a** with methanolic potassium hydroxide gave back **3a** whereas potassium tertiary butyrate in DMSO hydrolysed **3a** to **3** suggesting the hindered nature [7] of the carboxyl group possibly at C-17 position

The acid **3** and its derivatives **3a** and **3b** showed positive Liebermann–Burchardt tests for triterpenes and they developed a yellow colour with TNM Perbenzoic acid titration indicated the presence of only one double bond in sebiferenic acid, which was non-reducible with H_2–Pd on charcoal Periodic acid titration of **3a** showed that the two hydroxyl groups were vicinal to each other, CrO_3–pyridine oxidation of **3a** at 0° furnished a diketone **3c** (⇌diosphenol **3d**), $C_{31}H_{46}O_4$, mp 180–182°, λ_{max}^{EtOH} 272 nm (log ε 3 68), λ_{max}^{NaOH} 324 nm (log ε 3 56), ν_{max}^{nujol} cm^{-1} 3440 (OH), 1730 (COOMe), 1660, 1640 (α, β-unsaturated ketone), 820

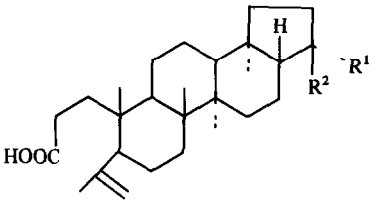
The 80 MHz ^{1}H NMR spectrum of **3b** showed the presence of seven tertiary methyl groups on saturated carbons at δ 0 85 (3H, *s*), 0 93 (15H, *s*) and 1 05 (3H, *s*), two acetoxy methyls at 1 98 and 2 04 (6H, 2*s*) and one carbomethoxy group at 3 58 (3H, *s*) One of the two protons geminal to the acetoxy groups appeared as an unsymmetrical doublet at δ4 7 (1H, J = 10 Hz) and another as a doublet of a triplet at 5 05 (1H, J = 10 Hz, 10 5 Hz) and a proton on trisubstituted double bond having vicinal and allylic protons at 5 50 (1H, *m*, $W_{1/2}$ = 8 Hz) Assuming that the unsymmetrical doublet at δ4 7 is due to the axial proton attached to C-3 containing the acetoxy group in the equatorial position as in most triterpenoids, the coupling constant (J = 10 Hz) indicated that the vicinal proton at C-2 (δ5 05) is *trans*-axial confirming the position of the second acetoxy group at C-2 being equatorially oriented [8, 9] This showed the presence of 2α,3β-diol system in **3** as is observed in the case of baccatin [10]

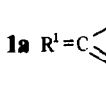
The position of the carboxyl group and the stereochemistry of the hydroxyl groups are further proved by acid isomerization of **3b** to acetyl methyl crategolate (**4**) This further showed that the acid belongs to the taraxerane skeleton

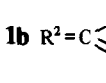
The position of the carboxyl group at C-17 and the double bond at Δ^{14} position have conclusively been proved by analysis of the mass spectra of **3a**, **3b** and **3c** The CI mass spectrum of **3a** showed peaks at *m/z* (rel int) 487 $[MH]^+$ (7), 469 $[MH-H_2O]^+$ (100), 451 $[469-H_2O]^+$ (86), 427 $[MH-COOMe]^+$ (20), 409 $[469-HCOOMe]^+$ (64), 391 $[409-H_2O]^+$ (10), 369 (3), 348 (2), 315 (10), 303 (19), 289 (17), 275 (10), 263 (53), 249 (30), 248 (43), 235 (7), 223 (30), 207 (35), 205 (35), 203 (20), 189 (27), 177 (10), 175 (5), and 149 (20), whereas **3b** showed peaks at 571 $[MH]^+$ (10), 511 $[MH-AcOH]^+$ (11), 451 $[511-AcOH]^+$ (100), 402 (3), 391 (10), 387 (10), 369 (3), 315 (10), 303 (4), 289 (5), 275 (3), 263 (16), 249 (20), 248 (17),

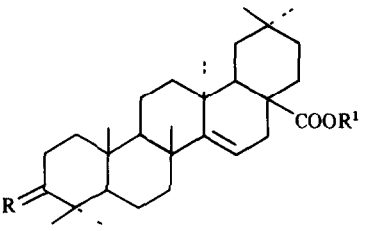
*Part 3 in the series "Chemical Investigation of the Bark of *Sapium sebiferum* Roxb" For Part 2 see ref [13]

†To whom correspondence should be addressed



1a R^1 =  , R^2 = H

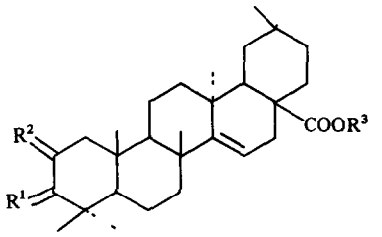
1b R^2 =  , R^1 = H



2 R =  , R^1 = H

2a R =  , R^1 = Me

2b R = O, R^1 = Me

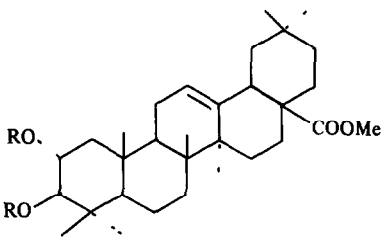


3 R^1 =  , R^2 =  , R^3 = H

3a R^1 =  , R^2 =  , R^3 = Me

3b R^1 =  , R^2 =  , R^3 = Me

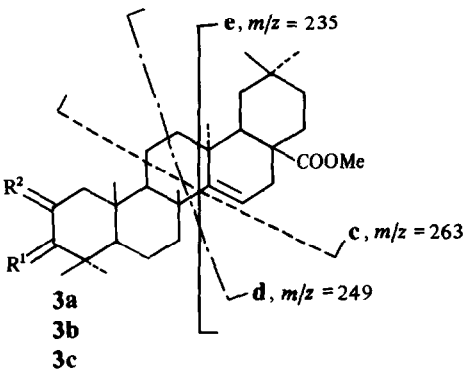
3c R^1 = O, R^2 = O, R^3 = Me



4 R = OAc

203 (10), 189 (36), 175 (5), and **3c** at 483 $[MH]^+$ (100), 467 (10), 423 $[MH - AcOH]^+$ (36), 369 (2), 355 (15), 315 (2), 303 (2), 289 (2), 275 (2), 263 (4), 249 (4), 248 (4), 203 (15), 189 (9), 165 (10), 151 (20), 121 (10), 107 (20) The appearance of identical peaks must be due to the formation of identical mass fragments from the compounds **3a**, **3b** and **3c** Formation of a few of them could be explained by assuming the presence of a carbomethoxy group at the C-17 position and a double bond at the Δ^{14} position of the taraxerane skeleton [2, 11]

The compounds **3a**, **3b** and **3c** showed the fragments **a** at *m/z* 318, 402 and 314 accompanied by ion **b** [**a** – Me] at 303, 387 and 299 respectively though of low intensity These fragments are typical of taraxerane Δ^{14} double bond [11] The common fragments at *m/z* 263, 249 and 235 may be due to fragments **c**, **d** and **e** formed by rupture of ring C which are accompanied by fragments **c'**, **d'** and **e'** each 60 mass units lower than **c**, **d** and **e**, respectively, and formed by loss of HCOOMe, as observed in the case of aleurotilic acid [2] Beside the above peaks, the mass spectrum of **3c** showed prominent fragments at *m/z* 355, 165, 151, 121 and 107 All the above data are compatible with structure **3** for sebiferenic acid A chemical correlation with a suitable member of the taraxerane series was achieved by auto-oxidation of methyl aleuritolonate (**2b**) which furnished a diketone identical with **3c** obtained by



3a
3b
3c

oxidation of **3a** A comparison of the ^{13}C NMR spectrum (Experimental) of **3b** with the spectra of aleuritolic acid derivatives [12] further confirmed the structure **3** of sebiferenic acid as 2α,3β-dihydoxytarxer-14-en-28-oic acid

EXPERIMENTAL

Mps are uncorr IR (nujol) Beckman IR-20, UV (EtOH) Beckman DU-2, ^1H NMR and ^{13}C NMR ($CDCl_3$) Ft-80A

Varian Spectrometer using TMS as internal standard, MS CH_4 chemical ionization method, alumina used for chromatography Sarabhai Mark, deactivated with 10% aq AcOH (4 ml/100 g Al_2O_3)

Extraction Dried and powdered bark of *Sapium sebiferum* Roxb was extracted with C_6H_6 in a Soxhlet The yellow insoluble solid (3,4-di-*O*-methyl ellagic acid) [13] was separated by filtration and the solvent distilled off under red pres The residue was extracted with Et_2O, shaken with 10% aq NaOH and the alkaline layer separated from the neutral layer The alkaline layer was cooled, acidified with 10% aq HCl and extracted with Et_2O The solvent was distilled off and the residue (10 g) absorbed on alumina (400 g) and then eluted with solvent mixtures of increasing polarity

Isolation of sebiferic acid (**1b**) The C_6H_6–petrol (4 1) eluate on crystallization from MeOH furnished an amorphous solid (0 3 g), mp 176–180°, which was esterified with CH_2N_2 to afford the methyl ester of **1b**, mp 134–136° This was found to be identical with an authentic sample (mmp co-TLC and co-IR)

Isolation of aleuritolic acid (**2**) The C_6H_6–Et_2O (9 1) eluate (0 1 g) on crystallization (MeOH) furnished an amorphous solid of **2**, mp 298–299°, IR ν_{max}^{nujol} cm^{-1} 3400 (OH), 1700 (COOH), 820 (>C=C<$_H$) [Found C, 79 30, H, 10 35 Calc for $C_{30}H_{48}O_3$ C, 78 94, H, 10 52%]

Methyl aleuritolate (**2a**) The Et_2O soln of **2** when esterified with CH_2N_2 gave **2b**, mp 207–209°, IR ν_{max}^{nujol} cm^{-1} 3350 (OH), 1735 (COOMe), 820 (>C=C<$_H$), identical with an authentic sample of methyl aleuritolate (mmp, co-TLC and co-IR) [Found C, 78 90, H, 10 56 Calc for $C_{31}H_{50}O_3$ C, 79 10, H, 10 71%]

Isolation of sebiferenic acid (**3**) The most polar eluent C_6H_6–Et_2O (3 2) afforded a solid which on crystallization from MeOH furnished **3**, as an amorphous solid (0 2 g) mp 325° (dec), $[\alpha]_D^{CHCl_3}$ + 32° [Found C, 76 17, H, 10 20 $C_{30}H_{48}O_4$ requires C, 76 23, H, 10 24%]

Methyl sebiferenate (**3a**) An Et_2O soln of **3** was esterified with CH_2N_2 and the solid obtained after the usual work up procedure was chromatographed Elution with C_6H_6–Et_2O (9 1) gave a solid which on crystalization from $CHCl_3$–MeOH furnished **3a**, mp 254–255°, $[\alpha]_D^{CHCl_3}$ + 15° [Found 3, 76 46, H, 10 30 $C_{31}H_{50}O_4$ requires C, 76 50, H, 10 35%]

Acetyl methyl sebiferenate (**3b**) Compound **3a** when acetylated with Ac_2O–pyridine (afforded **3b**, mp 224–226°, $[\alpha]_D^{CHCl_3}$ + 12°, ^{13}C NMR (20 MHz, $CDCl_3$) δ 16 47 (*q*, C-25), 17 37 (*t*, C-11), 17 46 (*q*, C-24), 18 58 (*t*, C-6), 20 70 (*q*, $C_2\alpha$-OCO<u>C</u>H$_3$), 21 02 (*q*, $C_3\beta$-OCO<u>C</u>H$_3$), 22 35 (*q*, C-30), 26 14 (*q*, C-27), 28 29 (*q*, C-26), 29 66 (*q*, C-23), 29 21 (*s*, C-20), 30 96 (*t*, C-16), 31 65 (*t*, C-12), 32 07 (*t*, C-22), 33 25 (*q*, C-29), 33 75 (*t*, C-21), 35 46 (*t*, C-7), 37 34 (*s*, C-13), 38 94 (*s*, C-10), 39 25 (*s*, C-4), 40 80 (*t*, C-19), 41 86 (*d*, C-18), 43 24 (*t*, C-1), 49 05 (*d*, C-9), 51 25 (*s*, C-17), 51 59 (*q*, COO<u>C</u>H$_3$), 55 23 (*d*, C-5), 69 96 (*d*, C-2), 80 62 (*d*, C-3), 116 79 (*d*, C-15), 160 10 (*s*, C-14), 170 31 (*s*, $C_3\beta$-O<u>C</u>OCH$_3$), 170 57 (*s*, $C_{2\alpha}$-O<u>C</u>OCH$_3$) and 178 25 (*s*, C-28) [Found C, 73 60; H, 9 56 $C_{35}H_{54}O_6$ requires C, 73 65, H, 9 54%]

Hydrolysis of **3a** (*i*) Compound **3a** was refluxed (4 hr) with methanolic KOH (10%), and after the usual work up **3a** was recovered (*ii*) Compound **3a** dissolved in DMSO was refluxed (4 hr) with 1 N *t*-BuOK in *t*-BuOH After usual work up and crystallization ($CHCl_3$–MeOH) an amorphous solid was obtained, mp 325°, identical (mmp and co-IR) with authentic **3**

Oxidation of **3a** *to* **3c** Compund **3a** (0 1 g) in pyridine (1 ml) was treated with CrO_3 in pyridine below 5° for 12 hr After the usual work up the solid was chromatographed and elution with C_6H_6–petrol (2 3) furnished diketone **3c** (⇌**3d**, green colour with neutral $FeCl_3$), mp 180–182° [Found C, 77 10, H, 9 63 $C_{31}H_{46}O_4$ requires C, 77 14, H, 9 61%]

Auto-oxidation of **2b** Treatment of **2a** with CrO_3–pyridine at room temp yielded methyl aleuritolonate (**2b**), mp 174–175°, IR ν_{max}^{nujol} cm^{-1} 1735 (COOMe), 1705 (>C=O), 820 Compound **2b** (0 1 g) suspended in a mixture of *t*-BuOK in *t*-BuOH (10 ml) was stirred in a stream of O_2 for 2 hr The mixture was diluted with H_2O, acidified with aq HCl, extracted with Et_2O and the solvent removed The residue on chromatography and elution with C_6H_6–petrol (2 3) afforded a solid, which crystallized from $CHCl_3$–MeOH, mp 180–182°, identical with **3c** (mp, co-TLC, green colour with neutral $FeCl_3$)

Isomerization of **3b** *to* **4** Compound **3b** (0 05 g) was isomerized in glacial AcOH (50 ml) by heating with conc HCl (2 5 ml) for 15 min The crystalline solid after usual work up from $CHCl_3$–MeOH furnished pure **4** (0 02 g), mp 166–168°, which was found to be identical with an authentic sample of acetyl methyl crategolate (mmp, co-IR, co-TLC)

Acknowledgements—We thank Dr A Duffield, Biomedical Mass Spectrometry Unit, The University of New South Wales, Kensington, Australia for the mass spectra, Dr S K Sengupta, EIPW, Calcutta for the Optical rotations, and Dr A Bernhardt, West Germany for the micro-analysis One of us (S De) is thankful to U G C, New Delhi for the award to a FIP Fellowship The authors thanks are also due to Dr T K Roy, Senior Research Asssistant, Dept of Chemistry, University of North Bengal for his valuable assistance and discussions

REFERENCES

1 Pradhan, B P and Khastgir, H N (1973) *Indian J Chem* **11**, 1217
2 Misra, D R and Khastgir, H N (1970) *Tetrahedron* **26**, 3017
3 Yosioka, I, Nakanishi, T and Ketagawa, I (1968) *Tetrahedron Letters* 1485
4 Nakanishi, T, Fuziwara, T and Tomita, K (1968) *Tetrahedron Letters* **12**, 1491
5 Yosioka, I, Nakanishi, T, Yamauchi, H and Ketagawa, I (1971) *Tetrahedron Letters* 1161
6 Nakanishi, T, Yamauchi, H, Fuziwara, T and Tomita, K (1971) *Tetrahedron Letters* 1157
7 Chakaborti, P, Mukherjee, D K, Barua, A K and Das, B C (1968) *Tetrahedron* **24**, 1107
8 Beaton, M and Spring, F S (1955) *J Chem Soc* 2131
9 Bacca, N S and Williams, D H (1964) *Application of NMR Spectroscopy in Organic Chemistry* Holden Day, San Francisco
10 Saha, B, Naskaer, D B, Misra, D R, Pradhan, B P and Khastgir, H N (1977) *Tetrahedron Letters* 3095
11 Budzikiewicz, H, Wilson, J M and Djerassi, C (1963) *J Am Chem Soc* **85**, 3688
12 Carpenter, R C, Sotheeswaran, S and Sultanbawa, M U S (1980) *Org Magn Reson* **14**, 462
13 Pradhan, B P and Khastgir, H N (1973) *Indian J Chem.* **11**, 1220