REFERENCES

1 Bhandari, P. and Rastogi, R. P. (1984) *Phytochemistry* **23**, 1699.
2. Budzikiewicz, H., Wilson, J. M. and Djerassi, C. (1963) *J. Am. Chem. Soc.* **85**, 3688.
3. Lehn, J. M. and Ourisson, G. (1962) *Bull. Soc. Chim. Fr.* 1137.
4. Williams, D. H. and Bhacca, N. S. (1964) *J. Am. Chem. Soc.* **84**, 2742.

Phytochemistry, Vol 23, No 9, pp 2085–2087, 1984.
Printed in Great Britain.

0031-9422/84 $3 00 + 0.00

LATEX EXTRACTABLES OF *CALOTROPIS GIGANTEA*

SWAPNADIP THAKUR, PRANTIKA DAS, TOSHIHIRO ITOH,* KAZUNORI IMAI* and TARO MATSUMOTO*

Department of Chemistry, University of Burdwan, Burdwan-713104, India; *College of Science and Technology, Nihon University, 1-8, Kanda Surugadai, Chiyoda-ku, Tokyo, 101 Japan

(*Revised received* 5 *March* 1984)

Key Word Index—*Calotropis gigantea*; Asclepiadaceae; latex; 3′-methylbutanoyl, acetyl and free α-amyrin, β-amyrin, ψ-taraxasterol, taraxasterol and lupeol; alkanes.

Abstract—The hexane and methanol soluble extract of the latex coagulum of *Calotropis gigantea* afforded two new triterpene esters, *viz.* 3′-methylbutanoates of α-amyrin and ψ-taraxasterol, besides the known 3′-methylbutanoates of three triterpene alcohols. The compositions of the alkane fraction, total triterpene alcohol fraction, and free, acetyl and 3′-methylbutanoyl triterpene alcohol fractions of the extract were determined.

INTRODUCTION

The milk-weed family (Asclepiadaceae) is very rich in latex bearing plants and a number of species have been investigated for the constituents of solvent extractables [1]. Although the leaves and root-bark of *Calotropis gigantea* have previously been examined [2–4], no attempt has yet been made to characterize the latex constituents of this plant. We have, therefore, undertaken here the detailed analysis of the latex constituents of *C. gigantea*.

RESULTS AND DISCUSSION

The coagulum of *C. gigantea* latex was extracted with hexane and methanol. The hexane extract was subjected to chromatography over silica gel which afforded a hydrocarbon fraction (2.5% of the hexane extract). The GLC analysis of the fraction showed the predominance of C_{14}, C_{16} and C_{18} alkanes with a consequently higher even : odd ratio (3:1). This is interesting since the alkane profile in cuticular lipids usually display a high odd : even ratio with maxima of C_{29}, C_{31} and C_{33}. The hexane and methanol extracts were combined and a portion of the hydrocarbon free mixture from the combined extracts was hydrolysed and subjected to silica gel TLC which afforded one major band corresponding to 3β-monohydroxy triterpene. The fraction from this band was acetylated and the composition was determined by GLC as shown in Table 1. The following five triterpenes were identified by GLC and GC/MS as the acetates (3β-OAc = **b**): α-amyrin (5α-urs-12-en-3β-ol, **1b**), β-amyrin (5α-olean-12-en-3β-ol, **2b**), ψ-taraxasterol (5α-taraxast-20-en-3β-ol, **3b**), taraxasterol [5α-taraxast-20(29)-en-3β-ol, **4b**], and lupeol [5α-lup-20(29)-en-3β-ol, **5b**].

The remaining portion of the hydrocarbon free triterpene mixture was separated into four major bands (referred to as bands 1–4 in order of polarity, beginning with the least polar) on silica gel TLC. Co-chromatographic studies with authentic triterpene acetate (**4b**) and free triterpene alcohol (**4a**; 3β-OH = **a**) indicated the occurrence of such components in bands 3 (R_f 0.58) and 4 (0.15), respectively. The IR spectra showed that the fractions from bands 1 (R_f 0.79), 2 (R_f 0.72) and 3 were the mixtures of esterified components (ν_{max} = 1720–1730 cm^{-1}). The components from band 2 (*ca* 30%) showed very long retention times on GLC, and GC/MS analysis revealed that the major five components were the esters (3β-$OCOC_4H_9$ = **c**) of C_5-saturated fatty acids with five triterpene alcohols (*m/z* 510, $[M]^+$; *m/z* 408, $[M - C_5H_{10}O_2]^+$), (**1c–5c**). The composition of this fraction was determined by GLC and is shown in Table 1. The fraction was further fractionated by silver nitrate–silica gel TLC which afforded four bands (referred to as bands 2-1 to 2-4 in order of polarity, beginning with the least polar). Band 2-1 (R_f 0.89) gave a mixture of the pentanoic acid esters of α-amyrin (**1c**) and β-amyrin (**2c**). The fractions from bands 2-2 (R_f 0.69), 2-3 (R_f 0.57) and 2-4 (R_f 0.50) were the pentanoic acid esters of ψ-taraxasterol (**3c**), taraxasterol (**4c**) and lupeol (**5c**), respectively. The pentanoic acid esters of **3c** and **4c** showed the methylene multiplet at δ2.17 (2H, H-2′), deshielded by the adjacent carbonyl group, and the isopropyl doublet at δ0.96 (6H, *J* = 6 Hz, H-4′, H-5′) in the ^1H NMR spectra besides the signals arising from the triterpene moieties which suggested that the pentanoic acid moiety was 3-

Table 1. Percent compositions of total triterpene alcohol, and free, acetyl and 3'-methylbutanoyl triterpene alcohol fractions of the latex extractables of *Calotropis gigantea*

Triterpene alcohol	% Composition			
	Total	Free (a)	Acetate (b)	3'-Methyl-butanoate (c)
α-Amyrin (1)	8.9	8.8	10.5	8.4
β-Amyrin (2)	11.5	15.0	15.2	12.5
ψ-Taraxasterol (3)	32.2	37.8	32.3	24.2
Taraxasterol (4)	39.8	27.1	34.4	41.9
Lupeol (5)	7.6	8.1	7.6	5.2
24-Methylenecycloartanol (6)	—	2.3	—	—
Unidentified	—	0.9	—	7.8

methylbutanoic acid. This was confirmed by the direct comparison with authentic 3'-methylbutanoates of ψ-taraxasterol (**3c**) and taraxasterol (**4c**). Since the GLC correlation of the five triterpene esters was almost consistent with that of the acetyl derivatives of the corresponding triterpenes, 3-methylbutanoic acid might be attributed as the acid moiety of the esters of the other three triterpenes, α- and β-amyrin and lupeol, for which ^{1}H NMR spectra were unavailable. The components of band 3 (*ca* 54%) of the silica gel TLC described above consisted of the acetyl derivatives of five triterpene alcohols (**1b–5b**). The components of band 4 (*ca* 7%) was a mixture of five triterpene alcohols (**1a–5a**) and 24-methylenecycloartanol (24-methylene-9β,19-cyclo-5α-lanostan-3β-ol, **6a**). Percent compositions of the components from bands 3 and 4 determined by GLC of the acetates are shown in Table 1. Identification of each component was supported by GC/MS analysis. The constituents of the minor fraction from the least polar ester band (band 1, *ca* 9%) remains undetermined.

The co-occurrence of the 3'-methylbutanoyl esters of five triterpene alcohols (**1c–5c**) is of importance from the phytochemical and biogenetic points of view. The occurrence of 3-methylbutanoic acid as an acid moiety in combination with triterpene alcohols is very rare in the plant kingdom. Only β-amyrin (**2c**) and lupeol (**5c**) 3'-methylbutanoates have been found in *Glossostelma carsoni* roots [5] and *Hoya bella* latex [6], while the presence of taraxasteryl 3'-methylbutanoate (**4c**) was indicated in the root-bark of *C. gigantea* [4]. To the best of our knowledge, this is the first report of the characterization of α-amyrin (**1c**) and ψ-taraxasterol (**3c**) 3'-methylbutanoates in the plant kingdom.

EXPERIMENTAL

Mps are uncorr. ^{1}H NMR spectra (100 MHz) were determined in $CDCl_3$ with TMS as internal standard. Low-resolution MS (70 eV, m/z > 100) were taken with a GC/MS (2% OV-17, 2 m × 3 mm i.d. glass column, 280°). High-resolution MS (70 eV) were recorded by means of a probe injection. IR spectra were taken in KBr. GLC of alkanes was carried out on a 10% SE-30 glass column (2 m × 1.6 mm i.d.) temp. programmed 170° to 300° at 5°/min GLC of triterpene esters were performed on a SCOT OV-17 glass capillary column (30 m × 0.3 mm i.d.) at 260°. RR_t were expressed relative to cholesteryl acetate. Silica gel TLC (0.5 mm) was developed ×2 with hexane–EtOAc (6:1). $AgNO_3$–silica gel (1:4) TLC (0.5 mm) was developed ×4 with CCl_4–CH_2Cl_2 (5:1). Acetylation in Ac_2O–pyridine and hydrolysis in 5% KOH–MeOH soln were performed at room temp. overnight. α-Amyrin (**1a**, RR_t = 1.84 as for the acetyl derivative), β-amyrin (**2a**, 1.65), ψ-taraxasterol (**3a**, 2.40), taraxasterol (**4a**, 2.50), lupeol (**5a**, 1.93) and 24-methylenecycloartanol (**6a**, 2.10) were used as the reference specimens [7]. The latex of *C. gigantea* (16 g) was collected locally in India in 1981 from the leaf-stem juncture of the plant directly into THF. The THF soluble material was coagulated and the coagulum (2.5 g) exhaustively extracted with hexane (12 × 100 ml) and then with MeOH (10 × 100 ml), to yield 1.75 g and 0.51 g of the extracts, respectively. The composition of the hydrocarbon fraction, which was separated from the hexane extract and consisted mainly of *n*-alkanes, was determined by GLC as follows: C_{13} (10.1 mole %), C_{14} (23.6), C_{15} (4.3), C_{16} (19.9), C_{17} (2.3), C_{18} (13.2), C_{19} (1.5), C_{20} (5.9), C_{21} (0.7), C_{22} (2.2), C_{23} (0.7), C_{24} (0.9), C_{25} (0.8), C_{26} (0.5), C_{27} (0.6), C_{28} (0.4), C_{29} (0.5), C_{30} (0.8), C_{31} (0.7), branched alkanes (10.4).

Preparation of the 3'-methylbutanoates of ψ-taraxasterol (**3c**) *and taraxasterol* (**4c**). 3-Methylbutanoic acid (isovarelic acid) was refluxed with excess of $SOCl_2$ for 3 hr and the remaining $SOCl_2$ was then removed by distillation which gave 3-methylbutanoyl chloride. Esterification of ψ-taraxasterol (**3a**) and taraxasterol (**4a**) with 3-methylbutanoyl chloride in pyridine under reflux for 3 hr afforded **3c** and **4c**, respectively. The GLC, MS and ^{1}H NMR data of authentic **3c** and **4c** were indistinguishable from those of the corresponding natural compounds described below.

Physical properties. α-Amyrin 3'-methylbutanoate (**1c**). RR_t = 3.46 in GLC; MS m/z (rel. int.): 510 $[M]^+$ (3), 409 (1), 408 (2), 290 (2), 270 (3), 257 (2), 218 (100), 203 (15), 189 (22). *β-Amyrin 3'-methylbutanoate* (**2c**). RR_t = 3.06; MS m/z (rel. int.): 510 $[M]^+$ (1), 409 (1), 408 (1), 290 (1), 270 (1), 257 (1), 218 (100), 203 (38), 189 (16). *ψ-Taraxasteryl 3'-methylbutanoate* (**3c**). Mp 208–212°; RR_t = 4.53; MS m/z (rel. int.): 510.4439 ($C_{35}H_{58}O_2$ requires 510.4434) $[M]^+$ (15), 495.4202 ($C_{34}H_{55}O_2$, 1), 409.3801 ($C_{30}H_{49}$, 8), 408.3779 ($C_{30}H_{48}$, 17), 393.3489 ($C_{29}H_{45}$, 8), 291.2308 ($C_{19}H_{31}O_2$, 5), 257.2274 ($C_{19}H_{29}$, 4), 229.1945 ($C_{17}H_{25}$, 6), 218.2027 ($C_{16}H_{26}$, 6), 205.1909 ($C_{15}H_{25}$, 10), 204.1835 ($C_{15}H_{24}$, 13), 203.1772 ($C_{15}H_{23}$, 14), 191.1781 ($C_{14}H_{23}$, 18), 189.1654 ($C_{14}H_{21}$, 100), 175.1480 ($C_{13}H_{19}$, 13), 161.1306 ($C_{12}H_{17}$, 10); ^{1}H NMR: δ0.73 (3H, *s*, H-28), 0.85 (6H, *s*, H-23, H-24), 0.88 (3H, *s*, H-25), 0.95 (3H, *s*, H-27), 0.99 (3H, *d*, J = 7 Hz, H-29), 1.04 (3H, *s*, H-26), 1.65 (3H, *s*, H-30), 4.5 (1H, *m*, H-3α), 5.3 (1H, *m*, H-21),

0.96 (6H, *d*, $J = 6$ Hz, H-4', H-5'), 2.17 (2H, *m*, H-2'). The ^{1}H NMR signals arising from the triterpene moiety of this ester and **4c** were assigned with the aid of the lanthanide-induced-shift techniques [unpublished results]. *Taraxasteryl 3'-methylbutanoate* (**4c**). Mp 200–204°; $RR_t = 4.73$; MS *m/z* (rel. int.): 510.4459 ($C_{35}H_{58}O_2$) $[M]^+$ (19), 409 (43), 407 (20), 393 (10), 291 (8), 257 (6), 229 (7), 218 (7), 205 (19), 204 (17), 203 (20), 191 (33), 189 (100), 175 (17), 151 (14); ^{1}H NMR: δ0.85 (9H, *s*, H-23, H-24, H-28), 0.87 (3H, *s*, H-25), 0.93 (3H, *s*, H-27), 1.02 (3H, *s*, H-26), 1.02 (3H, *d*, $J = 6$ Hz, H-29), 4.5 (1H, *m*, H-3α), 4.61 (2H, *m*, H-30), 0.96 (6H, *d*, $J = 6$ Hz, H-4', H-5'), 2.17 (2H, *m*, H-2'). *Lupeyl 3'-methylbutanoate* (**5c**). $RR_t = 3.64$; MS *m/z* (rel. int.): 510 $[M]^+$ (21), 495 (14), 409 (10), 408 (10), 393 (10), 365 (6), 217 (44), 203 (41), 189 (100), 175 (27), 161 (30)

Acknowledgements—We thank Professor Y. Ichinohe (Nihon University, Japan) for valuable comments and the C.S.I.R. (New Delhi, India) for the award of a senior research fellowship to one of us (P.D.).

REFERENCES

1. Adams, R. P., Balandrin, M. F., Hogge, L., Craig, W. and Price, S. (1983) *J. Am. Oil Chem. Soc.* **60**, 1315, and references cited therein.
2. Hill, E G. and Sirkar, A. P (1915) *J. Chem. Soc.* 1437.
3. Balakrishna, K. J., Murthi, R. B. P and Seshadri, T. R. (1946) *Proc Ind. Acad. Sci.* **22A**, 138.
4. Anjaneyulu, V. and Row, L. R. (1968) *Curr. Sci* **37**, 156.
5. Reichstein, P., Kaufmann, H., Stoecklin, W. and Reichstein, T (1967) *Helv. Chim. Acta* **50**, 2114.
6. Baas, W. J. and Neimann, G. J. (1979) *Z. Naturforsch. Teil. C.* **34**, 5
7. Itoh, T., Uetsuki, T., Tamura, T. and Matsumoto, T (1980) *Lipids* **15**, 407.

Phytochemistry, Vol 23, No 9, pp 2087–2088, 1984.
Printed in Great Britain

0031–9422/84 $3 00 + 0.00
Pergamon Press Ltd

SITOSTEROL 3-*O*-β-D-XYLOPYRANOSIDE FROM *BAUHINIA CANDICANS*

ADOLFO M. IRIBARREN and ALICIA B. POMILIO*

Departamento de Química Orgánica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Pabellón 2, Ciudad Universitaria, 1428 Buenos Aires, Argentina

(*Received* 5 *January* 1984)

Key Word Index—*Bauhinia candicans*; Leguminosae; structure elucidation; steroidal glycoside; sitosterol 3-*O*-β-D-xylopyranoside.

Abstract—A novel steroidal glycoside was isolated from aerial parts of *Bauhinia candicans*. Its structure was determined as sitosterol 3-*O*-β-D-xylopyranoside by chemical and spectral methods.

INTRODUCTION

In continuation of our work on *Bauhinia candicans* Benth. (Leguminosae; subfamily Caesalpinioideae), a medicinal plant from Argentina we now report the isolation and identification of a novel steroidal glycoside, sitosterol 3-*O*-β-D-xylopyranoside (**1**). We have previously described [1] the isolation and identification of Δ^5-sterols, flavonoid glycosides, sitosterol 3-*O*-β-D-glucopyranoside, D-pinitol and trigonelline from this plant.

RESULTS AND DISCUSSION

Upon chloroform percolation of the methanolic extract of *Bauhinia candicans* and further chromatographic purification of this material a fraction was obtained rich in steroidal glycosides. Since the separation of its components was not possible, the fraction was methylated to yield a mixture of compounds which exhibited considerable R_f differences. The main component (**1**) was not methylated while the other two minor glycosides gave methylated derivatives. Further purification of **1** was achieved by acetylation and column chromatography of the acetylated products.

Upon acid hydrolysis of the acetyl derivative **1a** the chloroform phase provided an aglycone that was identified as sitosterol by mass spectrometry and by capillary column GLC. The aqueous phase gave a sugar that was characterized as xylose by preparing its alditol acetate.

The ^{1}H NMR spectrum of **1a** showed the following signals: an olefinic proton (H-6) at δ 5.35 (*m*), Me-18 at 0.68 (*s*), Me-19 at 0.99 (*s*), Me-26 and Me-27 at 0.83 (*d*, $J = 7$ Hz), Me-29 at 0.84 (*t*, $J = 6.5$ Hz) and Me-21 at 0.92 (*d*, $J = 3$ Hz). These are the typical signals for sitosterol. The anomeric proton of the xylose (H-1') was present as a doublet at δ4.56 with a $J_{1',2'} = 8$ Hz due to axial–axial coupling thus showing that the xylose was β-linked to the aglycone. Protons 2' and 3' were superimposed triplets ($J_{aa} = J_{1',2'} = J_{2',3'} = J_{3',4'} = 8$ Hz) at 4.94 and 5.12, respectively. The H-4' appeared as a superimposed multi-

*Research member of the National Research Council of Argentina (CONICET). To whom any inquiries should be addressed.