

SERRATENE TRITERPENES FROM *PINUS ARMANDII* BARK

JIM-MIN FANG, WEI-YU TSAI and YU-SHIA CHENG*

Department of Chemistry, National Taiwan University Taipei, Taiwan 10764, Republic of China

(Received 19 July 1990)

Key Word Index *Pinus armandii*; Pinaceae; bark; triterpenes; serratene; nor-serratene.

Abstract—Five known serratene triterpenes, a nor-serratene and four new serratene derivatives have been isolated from the bark of *Pinus armandii*.

INTRODUCTION

Pinus armandii var. *mastersiana*, the armand pine, is an economically important conifer indigenous to the south west region of China [1]. We have recently reported flavonoids and stilbenes from the heartwood of this plant [2]. As a continuing study, we report here that serratene triterpenes are present in the bark of *P. armandii*. Serratenes have been mainly isolated from conifers (Pinaceae and *Pinus* species) and club mosses (*Lycopodium* species) [3, 4]. They are a group of naturally occurring pentacyclic triterpenes biogenetically related to α -onocerin [5]. The characteristic structural features of serratene triterpenes incorporate seven tertiary methyl groups (instead of eight methyls in common pentacyclic triterpenes), a central seven-membered C ring and a double bond between C-14 and C-15.

RESULTS AND DISCUSSION

The bark of *P. armandii*, collected in the Central Range of Taiwan, was exhaustively extracted with acetone. The concentrated extract was subjected to chromatography to give nine serratene triterpenes (1–9) in addition to a nor-serratene (10). Compound 1, $C_{30}H_{50}O_2$ ($[M]^+$ m/z 442.3793), is identified as serrat-14-en-3 β ,21 α -diol [6]. The mass spectrum of 1 gave diagnostic intense fragments at m/z 207 (89%) and 220 (78%) resulting from cleavage of the seven-membered C ring, while fragments from retro-Diels–Alder cleavage are minor [7]. On the basis of NMR evidence, both 3 α -H (δ 3.17, *dd*, $J = 10, 4$ Hz) and 21 β -H (δ 3.22, *dd*, $J = 10, 4$ Hz) are shown to be axial. Thus, rings A and E are considered to be in chair conformations with equatorially oriented 3 β and 12 α hydroxy groups.

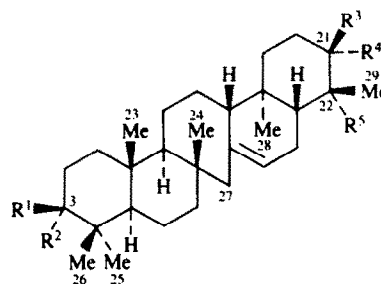
Compounds 2 (mp 305–308 $^\circ$) and 3 (296–298 $^\circ$), both having $[M]^+$ at m/z 442, are recognized as isomers of 1. The H-3 in compound 2 appears to be axial (on the α -face) as inferred from its NMR resonance (δ 3.16, *dd*, $J = 11, 5$ Hz), whereas the H-21 is equatorial (on the α -face) occurring at δ 3.43 as a broad singlet. On the other hand, both 3 β and 21 α protons in 3 are equatorial as their NMR signals appear at δ 3.37 and 3.43 as broad singlets. Thus, 2 and 3 are assigned as serrat-14-en-3 β ,21 β -diol and serrat-14-en-3 α ,21 β -diol, respectively [8].

Compound 4, $C_{31}H_{52}O_2$ (exact mass measurement, $[M]^+$ at m/z 456.3988) is a monomethoxy ether of 1,

which shows resonances at δ 3.33 (s) and 2.60 (*dd*, $J = 12, 4$ Hz) attributable to the methoxy group and the axial geminal proton. As the ^{13}C signals for carbons in the A ring of 4 are substantially different from those of 1, but carbons in the E ring remain unchanged (Table 1), the methoxy group is ascribed to the 3 β position. Thus, compound 4 (mp 318–319 $^\circ$) is identified as 3 β -methoxyserrat-14-en-21 α -ol [8].

Compound 5, $[M]^+$ at m/z 498, isolated for the first time from a natural source, shows IR absorption at 1720 cm^{-1} and 1H NMR resonance at δ 2.02 (3H, s) ascribed to an acetoxy group. This compound is 3 β -methoxy-21 α -acetoxyserrat-14-ene, an acetate of 4, by chemical correlation.

Compound 6, $[M]^+$ at m/z 454, contains a carbonyl group as indicated by the IR absorption at 1706 cm^{-1} . The structure can be determined as 3 β -methoxyserrat-14-en-21-one [8] by analysis of its NMR spectra using the pulse techniques of DEPT, 1H - ^{13}C one-bond heteronuclear COSY and 1H - 1H homonuclear COSY. According



	R ¹	R ²	R ³	R ⁴	R ⁵
1	OH	H	H	OH	Me
2	OH	H	OH	H	Me
3	H	OH	OH	H	Me
4	OMe	H	H	OH	Me
5	OMe	H	H	OAc	Me
6	OMe	H	=O		Me
7	OMe	H	=O		CH ₂ OH
8	OMe	H	=O		CHO
9	OMe	H	H	OAc	CHO
10	OMe	H	=O		H

Table 1. ^{13}C NMR spectral data of serratenes 1–10 (CDCl_3 , 75 MHz, δ)

C	1	2	3	4	5	6	7	8	9	10
1	38.6	38.6	38.6	38.5	38.5	38.5	38.5	38.4	38.5	38.5
2	25.2	25.2	25.2	22.4	22.3	22.3	22.4	22.3	22.4	22.3
3	78.8	78.8	76.1	88.5	88.5	88.4	88.4	88.4	88.4	88.4
4	38.2	38.2	37.7	38.2	38.2	38.2	38.2	38.2	38.2	38.2
5	55.7	55.7	49.4	56.3	56.3	56.3	56.0	56.2	55.6	56.3
6	18.9	18.9	18.8	18.8	18.8	18.7	18.7	18.7	18.6	18.7
7	45.1	45.2	44.9	45.2	45.2	45.2	45.2	45.1	45.1	45.2
8	39.0	39.0	38.2	38.9	38.9	38.9	38.9	38.9	38.9	38.9
9	57.1	56.8	56.9	57.2	57.0	56.4	56.3	55.0	56.3	54.4
10	36.1	36.0	35.9	36.1	36.0	36.1	36.1	36.6	36.1	36.1
11	25.4	25.4	25.4	25.3	25.3	25.5	25.7	25.7	25.7	25.7
12	27.6	27.2	28.4	27.7	27.2	27.2	27.3	27.2	27.2	27.3
13	62.8	62.9	62.7	62.9	62.9	62.8	62.7	62.6	62.8	62.7
14	138.2	138.5	138.6	138.3	138.3	138.3	138.5	138.6	138.1	138.6
15	122.2	122.0	122.0	122.1	121.9	121.9	121.7	120.8	121.3	121.6
16	24.0	24.0	24.0	24.1	24.1	24.4	24.6	24.6	24.4	29.5
17	49.5	43.4	43.4	49.5	49.6	51.2	52.2	53.6	52.0	49.3
18	38.9	37.1	37.3	38.9	37.1	37.1	37.1	37.1	37.1	37.1
19	29.2	31.2	31.2	29.7	36.7	38.3	37.1	38.7	36.0	39.0
20	27.2	27.5	27.1	27.2	23.8	34.7	35.4	37.1	24.2	38.0
21	79.2	76.2	76.2	79.2	81.1	216.9	216.7	209.4	78.9	213.3
22	37.1	37.4	37.4	37.1	37.7	47.6	52.8	63.2	52.5	45.6
23	15.7	15.7	15.6	15.7	15.7	15.7	15.7	15.7	15.7	15.7
24	19.8	19.8	19.8	19.8	19.8	19.8	19.8	19.8	19.8	19.8
25	28.1	28.1	30.0	28.1	28.1	28.1	28.1	28.1	28.1	28.1
26	15.4	15.4	13.5	16.2	16.2	16.2	16.2	16.2	16.2	16.2
27	56.0	56.2	56.3	56.1	56.0	55.9	55.8	55.8	55.9	56.0
28	13.4	13.3	13.3	13.4	13.4	12.9	13.7	12.7	14.5	11.5
29	27.5	27.7	21.8	27.5	27.5	24.5	20.7	16.3	21.1	11.0
30	14.6	21.8	27.7	14.6	15.7	21.5	65.8	201.9	204.6	
OMe	—	—	—	57.5	57.5	57.4	57.5	57.5	57.5	57.5
OAc					21.3				20.2	
					171.0				170.5	

to the octant rule [9], a negative Cotton effect in the CD spectrum of **6**, $[\theta]_{286} - 5000$ (MeOH; $c 7.5 \times 10^{-3}$), confirms the presence of a carbonyl group at C-21.

Compounds **7** ($\text{C}_{31}\text{H}_{50}\text{O}_3$), **8** ($\text{C}_{31}\text{H}_{48}\text{O}_3$) and **9** ($\text{C}_{33}\text{H}_{52}\text{O}_4$) are novel serratene triterpenes. They show six tertiary methyl groups in their NMR spectra (Table 1). By meticulous analysis of the spectral data, compounds **7–9** are determined as 3β -methoxy-30-hydroxyserrat-14-en-21-one, 3β -methoxy-21-oxoserrat-14-en-30-al and 3β -methoxy-21 α -acetoxyserrat-14-en-30-al, respectively. To support the hydroxy group being on C-30 (R^5) in **7** but not on C-29, ^1H ^1H long range COSY reveals interaction between H-20 α ($\delta 2.66$) and one of the CH_2OH ($\delta 3.57$). A long range interaction of H-17 and the aldehyde proton (CHO) in compound **8** was also observed. A negative Cotton effect in the CD spectrum further supports **8** having the aldehyde group on the α -face. The stereochemical relationship in compound **9** is similarly elucidated. The acetoxy group is equatorial because its geminal proton (H-21) occurs at $\delta 4.72$ as a double of doublets ($J = 11, 5$ Hz). As compound **9** also shows a negative Cotton effect, its aldehyde group is also on the α -face according to the exciton coupling theory [10].

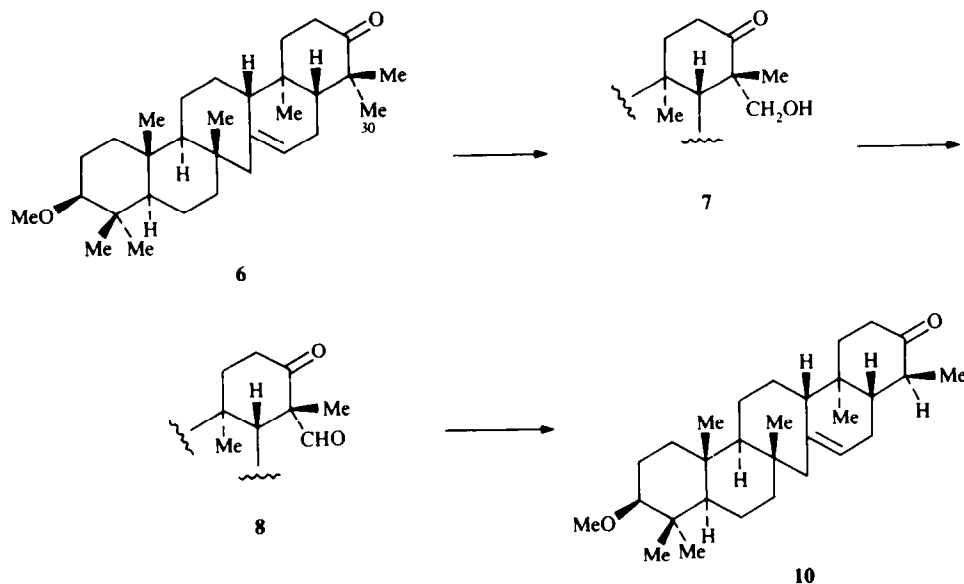
Compound **10**, $\text{C}_{30}\text{H}_{48}\text{O}_2$ ($[\text{M}]^+ m/z 440.3629$), $[\alpha]_{\text{D}} - 4.9^\circ$ (CHCl_3 ; $c 1.4$), is identified as an unusual norserra-

tene, 30-nor- 3β -methoxyserrat-14-en-21-one, by comparison of its spectral data with those of an authentic sample [11]. The absence of the 30-methyl group is consistent with a positive Cotton effect in the CD spectrum, $[\theta]_{282} 5400$ (MeOH; $c 8.5 \times 10^{-3}$).

The occurrence of 30-norserratene **10** ($\text{R}^5 = \text{H}$) is not yet fully understood. Finding the alcohol **7** ($\text{R}^5 = \text{CH}_2\text{OH}$) and the aldehyde **8** ($\text{R}^5 = \text{CHO}$) in the same plant source suggests that **10** may be biogenetically derived from serratene **6** ($\text{R}^5 = \text{Me}$) by a process of oxidative degradation via the intermediacy of **7** and **8** (Scheme 1).

EXPERIMENTAL

Plant material. Bark of *P. armandii* Franchet var. *mastersiana* Hayata was collected on Tayulin mountain (2600 m altitude), Taichung, in August 1985. A voucher specimen, identified by Dr. Ta-Wei Hu, has been deposited in the Herbarium of the Taiwan Forestry Institute. The bark was air-dried (760 g) and extracted $\times 3$ with Me_2CO . The combined extracts (37 g) were subjected to CC on silica gel (380 g) by elution with the gradients of hexane and EtOAc. The components of each frs were combined for further purification by HPLC in either normal phase (μ -Porasil or LiChrosorb Si 60) or reverse phase (LiChrosorb RP-18)



Scheme 1. Possible biogenesis of 30-nor-serratene (10).

modes to give serratenes **5** (13 mg), **6** (20 mg), **10** (28 mg), **9** (26 mg), **8** (29 mg), **4** (3.1 mg), **7** (13.3 mg), **1** (3 mg), **2** (800 mg) and **3** (6.2 mg), according to the ascending order of polarity.

3 β -Methoxy-21 α -acetoxyserrat-14-ene (5). C₃₃H₅₄O₃, crystals, mp 288.5–291.5° [α]_D 23.6° (c 1.0; CHCl₃). MS (70 eV) *m/z* (rel. int.): 498 [M]⁺ (9), 483 (4), 438 [M – HOAc]⁺ (3), 284 (3), 269 (10), 262 (28), 247 (5), 221 (83), 189 (68), 43 (100). HRMS: [M]⁺ at *m/z* 498.4044 (calcd 498.4072). IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 1720, 1250, 1103. ¹H NMR (CDCl₃, 300 MHz): δ 0.66 (s, Me-28), 0.72 (s, Me-26), 0.77 (s, Me-23), 0.78 (2H, *m*, H-5, H-13), 0.79 (s, Me-24), 0.82 (s, Me-29), 0.84 (1H, *m*, H-1), 0.87 (s, Me-30), 0.92 (s, Me-25), 1.10 (1H, *m*, H-12), 1.14 (2H, *m*, H-11, H-20), 1.18 (1H, *m*, H-7), 1.34 (*m*, H-17), 1.38 (2H, *m*, H-2, H-7), 1.46 (2H, *m*, H-6), 1.50 (2H, *m*, H-19), 1.60 (1H, *m*, H-20), 1.69 (1H, *m*, H-11), 1.73 (1H, *m*, H-27), 1.78 (3H, *m*, H-1, H-2, H-9), 1.97 (1H, *m*, H-12), 2.02 (s, MeCO₂), 2.02 (2H, *m*, H-16), 2.20 (1H, *br d*, *J* = 15 Hz, H-27), 2.60 (*dd*, *J* = 12, 4 Hz, H-3), 3.33 (s, OMe), 4.48 (*dd*, *J* = 11, 5 Hz, H-21), 5.30 (*br s*, H-15). Compound **5** was obtained as the exclusive product from acetylation (Ac₂O, pyridine, 12 hr at room temp.) of 3 β -methoxyserrat-14-en-21 α -ol (4).

3 β -Methoxy-30-hydroxyserrat-14-en-21-one (7). C₃₁H₅₀O₃, crystals, mp 226–228°. [α]_D –25.9° (c 0.89; CHCl₃). MS (12 eV) *m/z* (rel. int.): 470 [M]⁺ (10), 452 [M – H₂O]⁺ (5), 440 (56), 408 (12), 323 (22), 234 (13), 221 (100), 219 (4), 204 (75), 189 (25). HRMS [M]⁺ at *m/z* 470.3769 (calcd 470.3760). IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 3441, 1701, 1102. ¹H NMR (CDCl₃): δ 0.73 (s, Me-26), 0.76 (*m*, H-5), 0.78 (s, Me-23), 0.78 (*m*, H-13), 0.81 (s, Me-24), 0.85 (s, Me-28), 0.86 (1H, *m*, H-1), 0.93 (s, Me-25), 1.10 (1H, *m*, H-11), 1.14 (1H, *m*, H-12), 1.15 (s, Me-29), 1.18 (1H, *m*, H-7), 1.38 (2H, *m*, H-2, H-7), 1.44 (*m*, H-6), 1.54 (1H, *m*, H-19), 1.72 (1H, *m*, H-11), 1.78 (5H, *m*, H-1, H-2, H-9, H-17, H-27), 2.02 (2H, *m*, H-12, H-16), 2.16 (1H, *m*, H-19), 2.20 (1H, *br d*, *J* = 15 Hz, H-27), 2.38 (1H, *ddd*, *J* = 14, 4, 4 Hz, H-20 β), 2.60 (*dd*, *J* = 11, 3 Hz, H-3), 2.66 (1H, *ddd*, *J* = 14, 14, 6 Hz, H-20 α), 3.33 (s, OMe), 3.57 (1H, *d*, *J* = 7 Hz, CH₂OH), 4.00 (1H, *d*, *J* = 7 Hz, CH₂OH), 5.33 (*br s*, H-15).

3 β -Methoxy-21-oxoserrat-14-en-30-al (8). C₃₁H₄₈O₃, crystals, mp 228–233° (decomp); [α]_D 5.8° (c 0.76; CHCl₃) [θ]₂₈₈ –4800 (MeOH; c 6.6 × 10⁻³). MS (12 eV) *m/z* (rel. int.): 468 [M]⁺ (7), 453 (7), 438 (8), 323 (46), 284 (3), 279 (15), 232 (4), 221 (100), 217 (13), 189 (40). HRMS: [M]⁺ 468.3621 (calcd 468.3604). IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 3427, 1705, 1102. ¹H NMR (CDCl₃): δ 0.72 (s, Me-26), 0.76 (*m*, H-5), 0.77 (s, Me-23), 0.80 (s, Me-24), 0.82 (*m*, H-13), 0.84 (s, Me-28), 0.84 (1H, *m*, H-1), 0.93 (s, Me-25), 1.08 (1H, *m*, H-11), 1.18 (1H, *m*, H-7), 1.22 (1H, *m*, H-12), 1.23 (s, Me-29), 1.38 (2H, *m*, H-2, H-7), 1.46 (3H, *m*, H-6, H-19), 1.78 (5H, *m*, H-1, H-2, H-9, H-11, H-27), 1.90 (3H, *m*, H-12, H-16, H-17), 2.22 (2H, *m*, H-19, H-27), 2.32 (1H, *m*, H-16), 2.42 (1H, *m*, H-20 β), 2.60 (*dd*, *J* = 12, 4 Hz, H-3), 2.73 (1H, *ddd*, *J* = 14, 14, 6 Hz, H-20 α), 3.33 (s, OMe), 5.34 (*br s*, H-15), 9.85 (s, CHO).

3 β -Methoxy-21 α -acetoxyserrat-14-en-30-al (9). C₃₃H₅₂O₄, crystals, mp 230–232° (decomp.). [α]_D –8.3° (c 0.2; CHCl₃). [θ]₂₈₆ –1600 (MeOH; c 3.9 × 10⁻³). MS (12 eV) *m/z* (rel. int.): 512 [M]⁺ (17), 452 [M – HOAc]⁺ (50), 424 (50), 323 (25), 284 (9), 279 (25), 276 (6), 269 (6), 221 (100), 189 (42). HRMS [M]⁺ at *m/z* 512.3852 (calcd 512.3866). IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 1724, 1234, 1102 cm⁻¹. ¹H NMR (CDCl₃): δ 0.61 (s, Me-28), 0.73 (s, Me-26), 0.78 (s, Me-23), 0.75 (*m*, H-5), 0.78 (*m*, H-13), 0.80 (s, Me-24), 0.89 (1H, *m*, H-1), 0.93 (s, Me-25), 1.04 (s, Me-29), 1.08 (*m*, H-11), 1.13 (1H, *m*, H-12), 1.18 (1H, *m*, H-7), 1.25 (1H, *m*, H-19), 1.32 (1H, *m*, H-19), 1.38 (2H, *m*, H-2, H-7), 1.44 (*m*, H-6), 1.63 (2H, *m*, H-16, H-17), 1.78 (4H, *m*, H-1, H-9, H-11, H-27), 2.02 (s, MeCO₂), 2.02 (2H, *m*, H-12, H-20), 2.18 (1H, *m*, H-16), 2.20 (1H, *br d*, *J* = 15 Hz, H-27), 2.60 (*dd*, *J* = 12, 4 Hz, H-3), 3.33 (s, OMe), 4.72 (*dd*, *J* = 11, 5 Hz, H-21), 5.25 (*br s*, H-15), 10.08 (s, CHO).

Acknowledgements—We are grateful to Dr Ta-Wei Hu (Department of Forestry, Chinese Culture University) for collection of plant material, to Dr Tian-Shung Wu (Providence College) for taking the CD measurements, to Dr Conner (Forest Products Laboratory, Wisconsin, U.S.A.) for the ¹H NMR spectrum of 30-

nor-3 β -methoxyserrat-14-en-21-one and to the National Science Council (Republic of China) for financial support.

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Phytochemistry, Vol. 30, No. 4, pp. 1336–1339, 1991
Printed in Great Britain

0031 9422/91 \$3.00+0.00
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AGAVESIDE C, A STEROIDAL GLYCOSIDE FROM *AGAVE CANTALA**

GIRISH C. UNIYAL, PAWAN K. AGRAWAL,† OM P. SATI‡ and RAGHUNATH S. THAKUR

Central Institute of Medicinal & Aromatic Plants, Lucknow 226016, India; ‡Department of Chemistry, University of Garhwal, Srinagar 246 174, India

(Received 8 August 1990)

Key Word Index—*Agave cantala*; Agavaceae; fruits; steroidal glycoside; agaveside C.

Abstract—A new steroidal glycoside, agaveside C, isolated from the fruits of *Agave cantala* was characterized as 3 β -{ α -L-rhamnopyranosyl-(1→2)- β -D-glucopyranosyl-(1→3)- β -D-glucopyranosyl-[β -D-xylopyranosyl-(1→4)- α -L-rhamnopyranosyl-(1→2)]- β -D-glucopyranosyl}-2 α -hydroxy-25R-5 α -spirostane on the basis of chemical degradation, ¹³C NMR spectroscopy and fast atom bombardment mass spectrometry.

INTRODUCTION

In continuation of our chemical studies on *Agave cantala* [1], we now report the isolation and structural elucidation of a steroidal glycoside, agaveside C (1).

RESULTS AND DISCUSSION

The methanol extract of the fruits of *A. cantala*, on repeated chromatographic purification on silica gel columns afforded agaveside C (1). The glycosidic nature

of compound 1 was indicated by the broad absorption bands at 3397 and 1074 cm⁻¹ in its IR spectrum and many resonances in the region δ 67–88 in its ¹³C NMR spectrum. Its spirostane skeleton was suggested by the occurrence of a resonance at δ 109.78 in its ¹³C NMR spectrum [2, 3] and supported by the IR absorption bands at 899 and 922 cm⁻¹. The relative intensities of the two IR bands (899 cm⁻¹ > 922 cm⁻¹) revealed 25R stereochemistry which was consistent with the ¹³C shielding data.

The 500 MHz ¹H NMR exhibited two singlets at δ 0.64 and 0.76 and four doublets ($J = 6.0$ Hz) at δ 0.75, 1.03, 1.06 and 1.07 corresponding to two tertiary methyl groups at C-18 and C-19, and two secondary methyls at C-21, C-27 and C-6 of the rhamnopyranosyl units. The anomeric proton signals were observed at δ 4.78, 4.80, 5.05, 5.08, 5.17 and 5.44 and were correlated with ¹³C resonances at δ 102.91, 104.67, 104.81, 105.45, 104.81 and 104.97 respectively in a one bond CH correlation experiment, thus

*Part 34 in the series 'Studies on Indian Medicinal Plants'. For Part 33 see Jain, D. C., Agrawal, P. K. and Thakur, R. S. (1990) *Planta Med.* **56** (in press).

CIMAP Publication No. 936.

†Author to whom correspondence should be addressed.