Phytochemistry, Vol 23, No 11, pp 2583–2587, 1984
Printed in Great Britain

0031–9422/84 $3 00 + 0 00

CAPSUGENIN, A DAMMARANE TRITERPENE FROM *CORCHORUS CAPSULARIS*

CHOUDHURY M HASAN, AMINUL ISLAM,* MAHTABUDDIN AHMED,† MOFIZUD-DIN AHMED† and PETER G WATERMAN‡

Department of Pharmacy, University of Dhaka, Dhaka-2, Bangladesh, *Bangladesh Jute Research Institute (Technology Division), Dhaka-7, †Department of Chemistry, University of Dhaka, Dhaka-2, ‡Phytochemistry Research Laboratories, Department of Pharmacy (Pharmaceutical Chemistry), University of Strathclyde, Glasgow Gl 1XW, U K

(*Received* 2 *March* 1984)

Key Word Index—*Corchorus capsularis*, Tiliaceae, dammarane triterpene, capsin, capsugenin

Abstract—From the leaves of *Corchorus capsularis* a new dammarane triterpene glycoside, capsin, has been isolated Capsin was identified as the 3-glucoside of 20,24-epoxy-3β,12β,25,30-tetrahydroxydammarane from spectral data Capsin was tentatively assigned the (20*S*, 24*S*)-configuration by comparison with data available for similar compounds One of the oxidation products of the aglycone appears to be a friedo-type derivative, formed by concerted methyl migration on decarboxylation of a C-30 carboxylic acid intermediate

INTRODUCTION

Of the forty species of *Corchorus* only two are grown as jute in Bangladesh, *C capsularis* L and *C olitorius* L The leaves of *C capsularis* are bitter and reputed to have medicinal value [1] A number of investigations of the leaves of this species have revealed the presence of sitosterol and its glucoside and a number of unidentified, bitter, glycosides, capsularone, corchorol and capsularol [2, 3] The roots of both *C capsularis* and *C olitorius* were found to contain three ursane triterpenes, corosin (= capsularone) (**1**) [4, 5], ursolic acid and corosolic acid (**2**) [6] In this paper we report the results of a reinvestigation of the leaves of *C capsularis* and the identification of a novel 12-oxygenated dammarane triterpene

RESULTS AND DISCUSSION

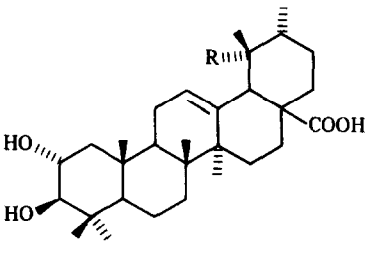
An alcoholic extract of the defatted leaves gave sitosterol glucoside, corosin (**1**) and a mixture of three bitter glycosides Of these the most polar was separated by preparative TLC and assigned the trivial name capsin (yield 0 13 %) Acid hydrolysis of capsin gave the aglycone capsugenin (**3**) which analysed for $C_{30}H_{52}O_5$ The mass spectrum obtained at 70 eV failed to show the true molecular ion, giving an apparent $[M]^+$ at *m/z* 474 ($C_{30}H_{50}O_4$) and a base peak at *m/z* 143 ($C_8H_{15}O_2$) that could be assigned to the hydroxyisopropyltetrahydrofuran ion (**4**) as seen in the trevoagenins [7] and betulafolientetraol [8] A low resolution EIMS obtained at 20 eV confirmed $[M]^+$ at 492

The ^{1}H NMR spectrum of **3** (Table 1) showed seven methyl groups and a number of deshielded protons A major feature was the AB*q* centred at δ 3 32 and 4 16 which could be assigned to a hydroxymethyl function derived from an original methyl substituent, a feature also seen in the trevoagenins [7, 9] A triplet at δ 3 37 was typical of the axial oxymethine (H-3) proton of triterpenes while a doublet of triplets at 3 53, showing two large and

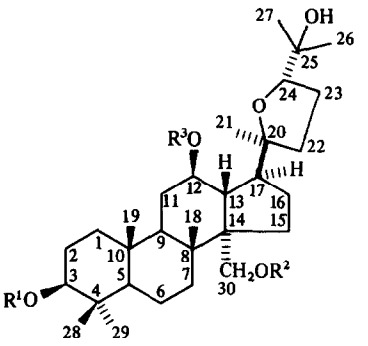
Table 1 ^{1}H NMR spectra of compound **3** and derivatives*

Proton	**3**	**5**	**6**	**7**
H-3	3 37 *t* (8)†	4 55 *dd* (8 4, 8 1)	4 55 *dd* (9 3, 7 2)	4 53 *dd* (8 4, 8 2)
H-12	3 53 *dt* (10 0, 5 0)	3 51 *dt* (10 2, 4 8)	4 80 *dt* (10 0, 5 8)	—
H-13	—	—	—	2 89 *d* (9 8)
H-17	—	2 24 *dt* (9 9, 6 2)	2 19 *br, s*	—
H-24	3 87 *t* (9)	3 86 *dd* (10 8, 4 8)	3 62 *dd* (8 8, 6 4)	—
H-18	1 10	1 09	1 09	1 24
H-19	0 97	0 98	0 98	0 96
H-21	1 20	0 99	0 99	1 19
H-26	1 20	1 22	1 16	—
H-27	1 27	1 26	1 17	—
H-28	0 89	0 89	0 89	0 99
H-29	0 82	0 91	0 91	0 74
H-30	3 32/4 16 ABq (11)	4 16/4 32 ABq (11 7)	4 11/4 31 ABq (11 7)	4 14/4 32 ABq (11 7)
Ac	—	2 02/2 05	1 99/2 01/2 05	2 02/2 06

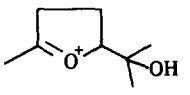
*All spectra run in $CDCl_3$ at 360 MHz except for **3** which was run at 100 MHz
†Coupling constants (Hz) are given in parentheses



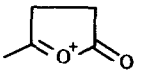
R
1 H
2 OH



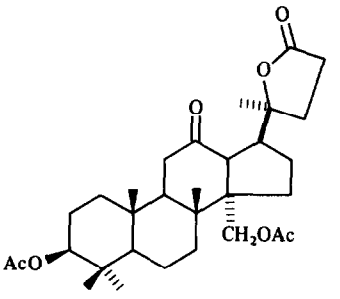
3 $R^1 = R^2 = R^3 = H$
5 $R^1 = R^2 = Ac$, $R^3 = H$
6 $R^1 = R^2 = R^3 = Ac$
13 R^1 = glucose, $R^2 = R^3 = H$



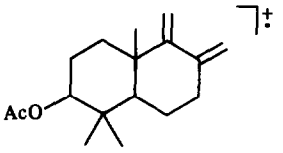
4



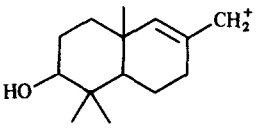
8



7



9



10

one small coupling indicated an axial oxymethine proton in the system $CH_2CH(OH)CH$ Another triplet centred at δ 3 87 was typical of H-24 in the tetrahydrofuran side-chain proposed [7, 8] One further feature of the spectrum was the occurrence of a hydroxyl signal as a sharp singlet at δ 5 69 This signal can be assigned to the 12-hydroxyl proton undergoing H-bonding to the oxygen of the furan ring [10] On the basis of the above capsugenin must possess four hydroxyl substituents, one primary, two equatorial secondary substituents and one tertiary, in the hydroxyisopropyltetrahydrofuran system

Acetylation of the aglycone at room temperature gave the diacetate (**5**) The ^{1}H NMR spectrum (Table 1) of **5** showed the anticipated shifts in the signals for H-3 and for the hydroxymethyl protons Acetylation under reflux gave the triacetate (**6**), the ^{1}H NMR spectrum of which showed additional deshielding for the H-12 proton The resistance of the 12-hydroxyl substituent to acetylation can be attributed to H-bonding to the furan In both compounds an additional resonance was visible at about δ 2 2, in **5** as a doublet of triplets and in **6** as a broad singlet This signal can be assigned to H-17 which, on the basis of coupling observed in **5**, must be axial and coupled to three protons, two at C-16 and one at C-13, thereby confirming that **3** was a triterpene of the dammarane type

Oxidation of the diacetate with Jones reagent gave **7**, which analysed for $C_{27}H_{44}O_7$ and gave a base peak in the EIMS for *m/z* 99 ($C_5H_7O_2$) indicative of ion **8** Facile loss of the hydroxyisopropyl group to leave a tetrahydrofuranone has been recorded in other triterpenes with this side-chain [7, 9, 11] The absence of the side-chain was confirmed by the ^{1}H NMR spectrum (Table 1) which revealed the loss of signals for two methyl groups and the H-24 proton The occurrence of a 5-membered lactone ring in **7** was also indicated by the IR spectrum (1770 cm^{-1}) In addition the signal for the H-12 oxymethine proton had disappeared indicating that **7** was a 12-oxo derivative, while signals for H-3 and the hydroxymethyl group remained Support for the assignment of a hydroxyl group to C-12 derives from (a) the presence of a new doublet at δ 2 89 in **7**, which must be assigned to the now deshielded H-13, coupling axial–axial with H-17, and (b) the very prominent *m/z* 262 fragment **9** originating via a C-11/C-12 cleavage that can only be rationalized if this

bond is α to a carbonyl The alternative hypothesis, arguing that the hydroxyl group was at C-16 and the δ2 89 resonance represented H-17 in the 16-oxo derivative can be discounted In both **5** and **7** the signal for the C-30 protons resonates at the same position (Table 1) whereas in 16,30-oxygenated dammarane derivatives the C-30 protons are deshielded by *ca* 0 2 ppm on conversion of the substituent at C-16 from hydroxyl to ketone [8, 12]

Placement of the primary alcohol at C-30 arises from the EIMS since the occurrence of **4** requires C-21, C-26 and C-27 to be unchanged while the fragments **10** from **3** and **9** from **7** require C-18, C-19, C-28 and C-29 to be present Further confirmation that the CH_2OH group must be at C-14 comes from the ^{1}H NMR spectrum The resonance at δ1 10 must be assigned to either the C-8 or the C-14 methyl group Its relatively deshielded position can only be justified by its assignment to C-8, where it will undergo 1,3-diaxial interaction with the C-10 methyl and consequently be deshielded [13]

^{13}C NMR spectra have been recorded for a number of dammarane derivatives [9, 14–16] and in the present work they were obtained for **3**, **5**, **6** and **7** (Table 2) Assignment of the triplet resonating at *ca* 65 ppm to the hydroxymethyl C-30 group was in close agreement with published data [9] The singlets found at *ca* 70 and 87 2 ppm are typical of C-25 and C-20 in all 20,24-epoxy-25-hydroxy dammaranes studied [16] The doublet at *ca* 70 ppm in **3** and **5** can be assigned to C-12, it exhibits a deshielding of about 5 ppm on acetylation to **6** with the anticipated shielding of C-11 (cf C-2 on acetylation at C-3) The large deshielding seen for C-22 in **6**, indicates that this carbon is within the deshielding cone of the acetyl carbonyl on C-12 The resonance at 209 8 ppm in the 12-oxo compound (**7**) is normal [17] for cyclohexanones and less than would have been anticipated if it had been due to a cyclopentanone (C-16 substitution) The C-3 carbon showed the expected deshielding in comparison to C-12 [16] and its occurrence at *ca* 80 ppm, rather than 76 ppm, together with the deshielded resonances for C-1, C-5 and C-29 were useful in confirming that the 3-hydroxyl substituent was equatorial The remaining deshielded doublet, found at *ca* 87 ppm in **3** and **5** can be placed at C-24 Finally the 176 6 ppm signal in the oxidation product **7** can be assigned to C-24 in the tetrahydrofuranone side chain [17]

On the above evidence the aglycone **3** can be characterized as 20,24-epoxy-3β,12β,25,30-tetrahydroxydammarane There remains the question of the stereochemistries at C-20 and C-24 The following evidence, whilst not conclusive, strongly supports the *S*-configuration at both these positions Conversion of **3** to **5** causes shielding of one methyl resonance from δ1 20 to 0 99 This can only be due to the anisotropic effect of the C-30 acetate on the C-21 methyl and this can only occur if the C-21 methyl lies below the plane of ring-D (in the α-position) Given that the furan ring is unable to rotate due to H-bonding to the 12-hydroxyl group this requires the C-21 methyl to be in the *S*-configuration (as in structure **11**) In **3** and **5** the C-25 methyl signals occur at δ1 20 and 1 27 Dammarane triterpenes with this side-chain and 20*R*-configuration [7, 11, 18] have C-25 methyl resonances that are equivalent or within 0 03 ppm of each other By constrast 20*S*-compounds have C-25 methyl resonances that are more deshielded and are quite strongly non-equivalent (0 07–0 13 ppm apart) [9, 18] Finally, the ^{13}C NMR resonances for C-24 in **3** and **5** (*ca* 87 5 ppm) are in accord with published data [16] for 24*S*-compounds whereas in 24*R*-compounds resonances for C-24 are generally found between 85 and 86 ppm

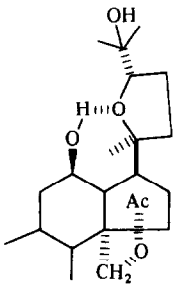
Table 2 ^{13}C NMR chemical shift values for compound **3** and derivatives*

Carbon	3	5	6	7
C-1	38 9	38 8	38 7	38 4
C-2	27 9	23 7	23 5	23 4
C-3	80 8	80 0	79 9	79 6
C-4	39 9	39 7†	39 5	40 3†
C-5	56 7	56 5	56 4	56 8
C-6	18 6	19 3	19 2	19 4
C-7	35 2	35 1	34 9	34 5
C-8	43 0	41 0†	41 2	41 1†
C-9	50 5	50 3	50 1	54 2
C-10	37 0	37 0	36 9	37 4
C-11	32 0†	31 8§	26 9	39 6
C-12	70 2	70 0	75 2	209 8
C-13	49 0	48 9‡	46 4	56 2
C-14	52 2	52 0	52 3	55 8
C-15	31 8†	31 6§	30 9	28 8
C-16	25 2	25 0	25 6	24 2
C-17	49 0	49 0‡	49 8	42 8
C-18	17 9‡	17 6‖	17 3‡	16 3‡
C-19	16 8‡	16 0‖	15 8‡	15 8‡
C-20	87 2	87 0	85 4	88 5
C-21	22 6	22 4	22 4	22 4
C-22	32 3†	32 1§	39 8	32 4
C-23	28 6	28 4	28 5	31 5
C-24	87 6	87 4	84 6	176 6
C-25	70 6	70 3	70 4	—
C-26	24 4	24 2	22 8†	—
C-27	29 0	27 9	27 5	—
C-28(α)	15 5‡	15 3‖	15 3‡	15 5‡
C-29(β)	28 0	27 8	24 0†	24 8
C-30	64 4	65 2	65 2	65 1
Ac		170 7	170 7	170 7
		170 3	170 3	170 3
		21 2	170 3	21 0
		21 0	21 7	21 0
			21 1	
			21 1	

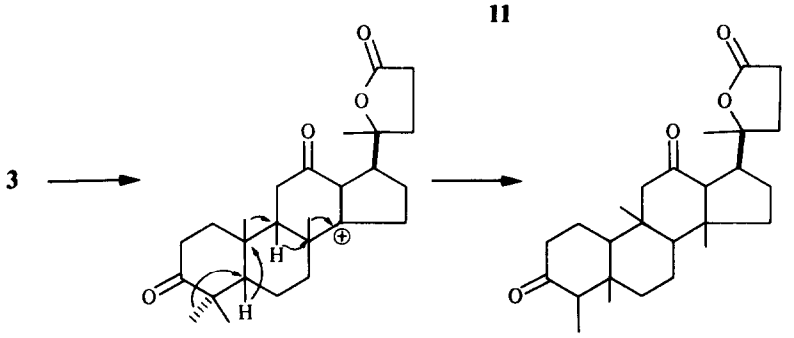
*Spectra run at 90 56 MHz in $CDCl_3$ Values in columns with identical superscripts are interchangeable

Oxidation of **3** gave four products, the major one of which analysed for $C_{26}H_{38}O_4$ The ^{1}H NMR spectrum showed five methyl substituents of which one was a doublet The ^{13}C NMR spectrum exhibited the signals for the furanone side-chain (also confirmed from EIMS) and had two carbonyl resonances as 213 7 and 211 2 ppm, attributable to C-3 and C-12 These data suggest the possible structure **12** for this compound which can be envisaged as being derived from **3** by oxidation and then decarboxylation at C-14 and a subsequent concerted 'friedo'-type methyl and hydride migration to give the methyl substitution pattern of a friedelane [19] Further work to substantiate this is in progress

The structure of capsin was established by the identification of glucose in the mother liquors after its hydrolysis to **3** Acetylation of capsin gave a pentaacetate in which only the pyranose sugar and C-30 were acetylated The



11



12

signal for the H-12 oxymethine proton was visible in the ^{1}H NMR spectrum at δ 3 70, similar to its position in the spectrum of **3** Capsin must therefore be the 3-glucopyranoside of capsugenin (**13**)

EXPERIMENTAL

Mps uncorr IR KCl discs NMR $CDCl_3$ with TMS as internal standard unless otherwise stated EIMS 70 eV, direct probe (180–200°)

Plant material Mature leaves of *C capsularis* were collected from Savar, Dhaka and air-dried

Extraction and isolation of triterpenes The powdered leaves (5 kg) were defatted by soaking in petrol (bp 40–60°) at room temp for 15 days and then extracted with 95% EtOH at room temp for 15 days The EtOH extract was filtered through activated charcoal (25 g l^{-1}) and the filtrate concd under red pres to an oil The oil was washed repeatedly with petrol, the residue dissolved in EtOH (2 l) and left overnight to yield a ppt of sitosterol glucoside (1 g) The mother liquor was concd and on standing gave **1** (2 g), identified by direct comparison with an authentic sample [6] The mother liquors were concd to a gum and triturated with EtOAc The resulting powder was dissolved in MeOH and repeatedly treated with activated charcoal until the soln was colourless On addition of a few drops of H_2O an amorphous solid (12 75 g) of the bitter glycosides was obtained

TLC of the bitter glycosides (silica gel, *n*-BuOH satd with H_2O) indicated the presence of three compounds, R_f 0 53, 0 73 and 0 78 Prep TLC of the mixture (500 mg) using the same system gave **13** (257 mg)

Capsin (**13**) Recrystallized from aq MeOH as needles, mp 210–212° $[\alpha]_D$ −16 4° (*c* 0 5, EtOH) IR ν_{max} cm^{-1} 3340 ^{1}H NMR (CD_3OD, 100 MHz) δ 0 93 (6H), 1 04 (3H), 1 20 (3H), 1 26 (9H) (7 × Me) Compound **13** (50 mg) in dry pyridine (3 ml) was treated with Ac_2O (2 ml) for 48 hr at room temp Normal work-up gave capsin acetate as an oil IR ν_{max} cm^{-1} 3400, 1730–1700 ^{1}H NMR (100 MHz) δ 0 87, 0 92, 0 94, 1 00, 1 12, 1 21, 1 24 (7 × Me), 1 98, 2 02 (6H), 2 04, 2 07 (5 × OAc), 3 36 (1H, *d*, *J* = 11 Hz, H-30), 3 70 (1H, *m*, H-12)

Hydrolysis of **13** *to yield capsugenin* (**3**) Capsin (450 mg) was dissolved in MeOH (17 5 ml) and conc H_2SO_4 (0 1 ml) added The mixture was refluxed for 6 hr, cooled, and pptd with excess H_2O The resulting solid was washed free of acid and recrystallized from aq MeOH as needles (235 mg), mp 230–232°, $[\alpha]_D$ −7 65° (*c* 1 3, EtOH) Found C, 72 9, H, 10 5 $C_{30}H_{52}O_5$ requires C, 73 2, H, 10 6 IR ν_{max} cm^{-1} 3335, 1240, 1025 ^{1}H NMR and ^{13}C NMR see Tables 1 and 2, respectively EIMS *m/z* (rel int) 474 $[M-H_2O]^+$ (2), 433 $[M-H_2O-C_3H_7O]^+$ (5), 415 (16), 397 (9), 207 (4), 205 (4), 191 (8), 189 (2), 175 (5), 143 $[C_8H_{15}O_2]^+$ (100), 125 (21), 121 (13), 107 (12), 95 (14), 93 (12), 85 $[C_5H_9O]^+$ (20)

Capsugenin diacetate (**5**) Compound **3** (250 mg) in dry C_5H_5N (4 ml) and Ac_2O (4 5 ml) was kept at room temp for 24 hr and following normal work-up gave **5**, recrystallized from aq MeOH as needles, mp 225–227°, $[\alpha]_D$ +2 55° (*c* 1 3, EtOH) IR ν_{max} cm^{-1} 3515, 3360, 1735 (OAc), 1710 (OAc), 1240, 1037 ^{1}H NMR and ^{13}C NMR see Tables 1 and 2, respectively EIMS *m/z* (rel int) 558 $[M-H_2O]^+$ (10), 517 (29), 499 (51), 439 (14), 379 (25), 249 (2), 191 (22), 189 (21), 187 (17), 159 (11), 147 (21), 143 (100), 133 (28), 125 (32), 121 (33), 95 (30), 85 (52)

Capsugenin triacetate (**6**) Compound **3** (100 mg) in dry C_5H_5N (5 ml) and Ac_2O (7 ml) was refluxed for 7 hr, the reaction mixture cooled and subjected to normal work-up to give **6**, recrystallized from aq MeOH as needles (40 mg), mp 137–138° IR ν_{max} cm^{-1} 3400, 1720, 1230, 1030 ^{1}H NMR and ^{13}C NMR see Tables 1 and 2, respectively

20,24-Epoxy-3β,30-diacetoxydammar-12,24-dione (**7**) Compound **5** (60 mg) in Me_2CO (10 ml) was treated with Jones' Reagent (5 ml) for 5 hr The reaction mixture was diluted with H_2O (30 ml) and extracted into Et_2O The extract was filtered, evaporated to dryness and the residue recrystallized from aq MeOH as needles (25 mg), mp 248–250° Found $[M]^+$ 530 3239, $C_{31}H_{46}O_7$ requires 530 3243 IR ν_{max} cm^{-1} 1770 (furanone), 1730, 1720, 1240, 1020 ^{1}H NMR and ^{13}C NMR see Tables 1 and 2, respectively EIMS *m/z* (rel int) 530 $[M]^+$ (44), 470 (12), 431 $[M-C_5H_7O_2]^+$ (49), 410 (23), 397 (19), 371 (10), 307 (12), 262 (33), 248 (23), 230 (10), 222 (15), 209 (29), 202 (34), 189 (15), 181 (60), 175 (17), 133 (22), 124 (56), 99 $[C_5H_7O_2]^+$ (100)

Compound **12**. Compound **3** (50 mg) in Me_2CO was treated with Jones' Reagent (5 ml) for 12 hr Work up of the reaction

mixture showed four compounds, R_f 0 15, 0 17, 0 22 and 0 31 (silica gel G, toluene–EtOAc–HOAc, 40 9 1) Prep TLC yielded the least polar band which recrystallized from MeOH as needles (10 mg), mp 285–290° (decomp) Found $[M]^+$ 414 2746, $C_{26}H_{38}O_4$ requires 414 2770 ^{1}H NMR (360 MHz) δ 0 73 (3H, *s*, Me) 1 00 (3H, *d*, J = 6 6 Hz, Me-4), 1 12, 1 24, 1 26 (3 × 3H, 3 × *s*, 3 × Me) ^{13}C NMR (90 56 MHz) 213 7, 211 2, 176 6, 88 5, 56 9, 56 0, 53 2, 52 0, 44 5, 42 8, 40 1, 39 9, 37 2, 37 0, 33 1, 32 4, 31 5, 29 6, 28 8, 24 9, 24 2, 22 3, 16 3, 15 9, 13 4, 11 6 EIMS *m/z* (rel int) 414 $[M]^+$ (24), 315 $[M - C_5H_7O_2]^+$ (14), 209 (13), 191 (12), 181 (33), 124 (37), 99 (100)

Acknowledgements—The authors wish to thank Dr I Sadler, Department of Chemistry, University of Edinburgh, for high-field NMR spectra (run on time allocated to P G W through S E R C) Additional NMR spectra on the glycoside and capsugenin and mass spectra were run by Dr R Anderson, Department of Chemistry and Molecular Biology, Swedish University of Agricultural Sciences, Upsala, Sweden and Dr P Bladon, Department of Pure and Applied Chemistry, University of Strathclyde Dr Abdul Matin Bhuiyan, Director, Technology Division, Bangladesh Jute Research Institute is gratefully acknowledged for providing laboratory space to one of us (A I.)

REFERENCES

1 Chopra, R N (1958) *Indigenous Drugs of India*, 2nd edn, p 501 Dhar & Sons, Calcutta
2 Saha, S and Choudhury, K N (1922) *J Chem Soc* 1044
3 Khuda, M Q, Khalique, A and Das, D C (1965) *Scientific Researches, East Regional Laboratories*, Vol 2
4 Khuda, M M and Islam, A (1971) *Pakistan J Sci Ind Res* **13**, 363
5 Khuda, M M and Hebermehl, K N (1974) *Z Naturforsch* **29C**, 209
6 Khuda, M M and Hebermehl, K N (1979) *Z Naturforsch* **34C**, 1320
7 Gonzalez, A G, Cortes, M and Suarez Lopez, E (1973) *An Quim* **69**, 817
8 Uvarova, N I, Malinovskaya, G V and Elyakov, G B (1976) *Tetrahedron Letters* 4617
9 Betancor, C, Cortes, M, Hernandez, R, Suarez, E, Prange, T and Pascard, C (1982) *Tetrahedron Letters* 1125
10 Shibata, S, Tanaka, O, Soma, K, Iida, Y, Ando, T and Nakamura, H (1965) *Tetrahedron Letters* 207
11 Warnoff, E N and Hall, C M M (1965) *Can J Chem* **43**, 3311
12 Kulshrestha, D K and Rastogi, R P (1973) *Phytochemistry* **12**, 887
13 Henrick, C A and Jefferies, P R (1964) *Aust J Chem* **17**, 915
14 Atopkina, L N and Uvarova, N I (1980) *Khim Prir Soedin* 205
15 Asakawa, J, Kasia, R, Yamasaki, K and Tanaka, O (1977) *Tetrahedron* **33**, 1935
16 Tanaka, O and Yahara, S (1978) *Phytochemistry* **17**, 1353
17 Wehrli, F W and Nishida, T (1979) *Fortschr Chem. Org Naturst* **36**, 70
18 Betancor, C, Freire, R, Hernandez, R, Suarez, E, Cortes, M, Prange, T and Pascard, C (1983) *J Chem Soc Perkin Trans* 1, 1119
19 Newman, A A (1972) *Chemistry of Terpenes and Terpenoids* Academic Press, London