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ISOLATION AND STRUCTURE OF TWO CARDIAC GLYCOSIDES FROM THE LEAVES OF *NERIUM OLEANDER*

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Key Word Index—*Nerium oleander*; Apocynaceae; *Nerium* leaves; cardiac glycosides; 3β-*O*-(D-diginosyl)-2α-hydroxy-8,14β-epoxy-5β-carda-16:17,20:22-dienolide; 3β-*O*-(D-diginosyl)-2α,14β-dihydroxy-5β-carda-16:17,20:22-dienolide.

Abstract—Two new cardiac glycosides, kaneroside and neriumoside, have been isolated from the fresh, undried, winter leaves of *Nerium oleander* and their structures established as 3β-*O*-(D-diginosyl)-2α-hydroxy-8,14β-epoxy-5β-carda-16:17,20:22-dienolide and 3β-*O*-(D-diginosyl)-2α,14β-dihydroxy-5β-carda-16:17,20:22-dienolide, respectively, through chemical and spectral studies.

INTRODUCTION

Nerium oleander Linn. (syn. *N. odorum*), distributed in the Mediterranean region and sub-tropical Asia, is indigenous to the Indo-Pakistan subcontinent. The plant is commonly known as 'Kaner' and its various parts are reputed to be therapeutic agents in the treatment of swellings, leprosy, eye and skin diseases. The leaves also possess cardiotonic and antibacterial properties and are a counter-poison against snakes [1, 2].

In view of its therapeutic properties, different parts of the plant have been subjected to chemical studies by various groups of workers, and several cardiac glycosides have been reported earlier [3]. The present paper deals with the isolation of two new cardiotonic glycosides, provisionally named as kaneroside (**1**) and neriumoside (**2**), from the fresh, uncrushed leaves of *N. oleander* (red-flowered variety). Their structures have been elucidated as 3β-*O*-(D-diginosyl)-2α-hydroxy-8,14β-epoxy-5β-carda-16:17,20:22-dienolide and 3β-*O*-(D-diginosyl)-2α,14β-dihydroxy-5β-carda-16:17,20:22-dienolide, respectively, through chemical and spectral studies.

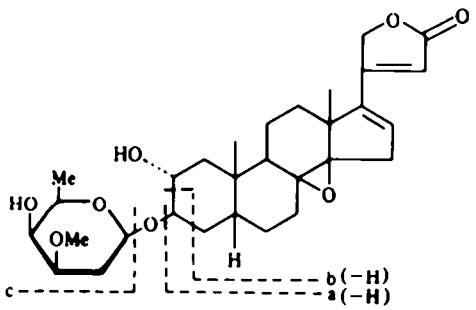
RESULTS AND DISCUSSION

Kaneroside (**1**) and neriumoside (**2**) were isolated from the neutral fraction of the methanolic extract of fresh, uncrushed *N. oleander* leaves employing classical methods of isolation followed by purification through preparative TLC, as described in the Experimental. The compounds gave positive tests for cardenolides (Legal and Raymond test) [4]. The molecular formula of kaneroside, $C_{30}H_{42}O_8$, was obtained through exact mass measurement of the molecular ion observed in the FAB mass spectrum. The IR spectrum showed peaks at 3450 (-OH), 1780, 1750 (β-substituted, α,β-unsaturated five-membered lactone) and 1625 cm^{-1} (>C=C). Its UV spectrum showed absorption at 267 nm, indicating the presence of a double bond conjugated with an α,β-unsaturated γ-lactone [5], which was placed at C-16 through the appearance of H-16 as a one-proton triplet at δ6.06 (J = 2.73 Hz), H-22 as a doublet of doublets at δ5.95 ($J_{22,21a}$ = $J_{22,21b}$ = 1.4 Hz) and two double doublets resonating at δ5.0 and 4.8 (J_{gem} = 16; $J_{21a,22}$ = $J_{21b,22}$ = 1.4 Hz, H-21a and H-21b) in the ^{1}H NMR spectrum.

The sugar molecule was indicated as D-diginose by the ^{1}H NMR (Table 1) and ^{13}C NMR (Table 2) spectral data of the glycosides [3]. Thus a one-proton doublet of doublets at δ4.56 ($J_{1\alpha,2\alpha}$ = 2.0, $J_{1\alpha,2\beta}$ = 9.75 Hz) was attributed to H-1′ while H-3′, H-4′ and H-5′ resonated as a three-proton multiplet between δ3.37 and 3.75. A three-proton doublet at δ1.33 (J = 7.1 Hz) was assigned to H-6′ while the methoxyl group located at C-3′ appeared at δ3.38 as a three-proton singlet.

The data recorded so far showed a close relationship of **1** with Δ^{16}-dehydroadynerigenin-β-D-diginoside [5]. The molecular formula of **1**, however, indicated that it had an additional hydroxyl function which could be located at C-2 since the ^{1}H NMR spectrum showed two sets of quartets at δ3.32 and 3.36 attributable to H-2 and H-3. Their coupling constants (J = 4.9 Hz) showed that the substituents at C-2 and C-3 have α and β dispositions, respectively, and the geminal protons (equatorial) are equally coupled with one axial and two equatorial protons. Placement of various functional groups in the steroidal skeleton left one oxygen function and a double-bond equivalent to be accounted for, which were taken for an epoxy function between C-8, C-14 on biogenetic grounds. This was confirmed through the absence of any other proton geminal to an oxygen function, the presence of two quaternary carbinylic carbons (δ65.1, C-8 and δ70.5, C-14) in the ^{13}C NMR spectrum and the formation of the polyene system (**3**) on hydrolysis, as observed in the case of Δ^{16}-dehydroadynerigenin [5].

Hydrolysis of **1** afforded **3** together with the sugar identified as diginose through paper chromatography. The molecular formula of **3** showed ten double-bond equivalents and four oxygen atoms in the molecule ($C_{23}H_{28}O_4$; high-resolution mass spectrometry). These observations together with the UV absorption maximum at 386 nm indicated that the hydrolysis was accompanied by opening of the epoxide ring, followed by dehydration resulting in the polyene system **3** as observed in the case of



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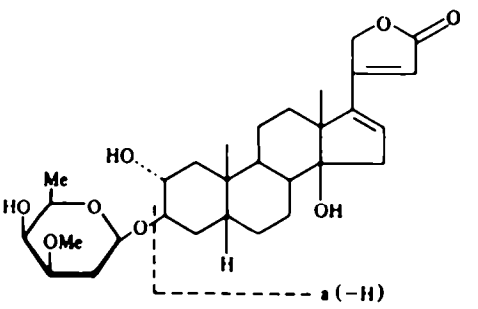

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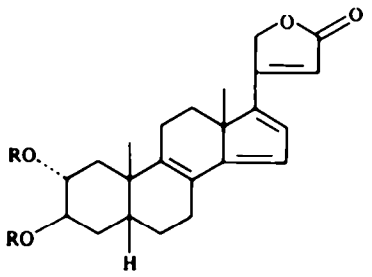
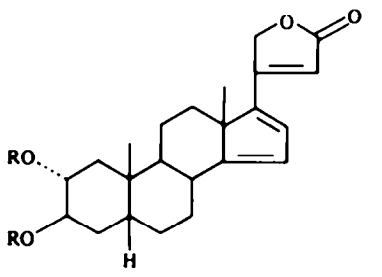


3 R = H
4 R = Ac



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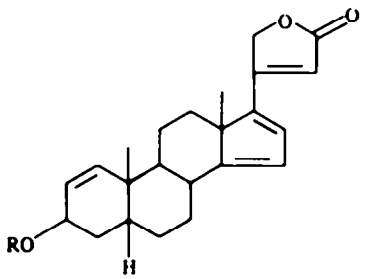
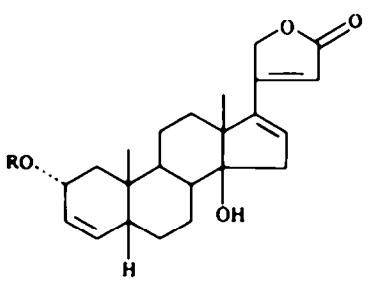
8 R = Ac



6 R = H
9 R = Ac



7 R = H

Δ^{16}-dehydroadynerigenin-*O*-diginoside [5]. Support for this came from the ^{1}H NMR spectrum (Table 1), which showed a one-proton doublet at δ6.70 (J = 2.37 Hz) for H-15 and a one-proton doublet at δ6.15 (J = 2.37 Hz) for H-16 together with the signals for the lactone ring protons and H-2 and H-3. On acetylation, 3 gave the diacetyl derivative (4) with molecular formula $C_{27}H_{32}O_6$ (high-resolution mass spectrometry). The ^{1}H NMR spectrum showed two sharp three-proton singlets at δ2.04 and 2.16 for the acetoxymethyl protons. Comparison of the chemical shifts (^{1}H NMR, ^{13}C NMR) of 1 with those of β-D-diginoside and the coupling constant observed for the anomeric protons [3] showed that the glycosidic linkage in 1 was β. Further, the general rule that the glycosidic linkages of sugars in the D- and L-series are β and α, respectively, [6, 7] and the observation that no enantiomer of diginose has been found in the genus *Nerium* [3] strongly suggested that the sugar moiety in 1 was D-diginose. In the light of the above discussion, structure 1 was assigned to kaneroside.

The molecular formula of neriumoside, $C_{30}H_{44}O_8$, was obtained through exact mass measurement of the molecular ion observed in the FAB mass spectrum. The IR spectrum showed peaks at 3450 (-OH), 1780, 1750 (β-substituted, α,β-unsaturated five-membered lactone) and 1625 cm^{-1} (>C=C). Its UV spectrum showed absorption at 267 nm, indicating the presence of a double bond conjugated with an α,β-unsaturated γ-lactone, which was placed at C-16 by the ^{1}H NMR spectrum (Table 1) in which H-16 appeared as a one-proton triplet at δ6.06 (J = 2.73 Hz), H-22 resonated as a one-proton doublet of doublets at δ5.95 ($J_{21,22}$ = 1.4 Hz) while H-21a and H-21b showed signals at δ4.92 and 4.96 (*dd*, J_{gem} = 16, $J_{21a,22}$ = $J_{21b,22}$ = 1.4 Hz). Two sets of quartets at δ3.32 and 3.36 with J = 4.9 Hz were assigned to carbinylic protons H-2 and H-3. The NMR spectral data (^{1}H and ^{13}C) indicated

Table 1. ^{1}H NMR spectral data for compounds 1–9

Protons	1	2	3	4	5	6	7	8	9
H-1	—					5.29 *d*•	—	—	5.29 *d*•
H-2	3.32† *ddd*‡	3.32† *ddd*‡	3.30 3.43 *m*	4.97–5.05 *m*	3.32 3.36 *m*	5.34 *m*	4.71† *m*	4.55–4.46 *m*	5.34 *m*
H-3	3.36† *ddd*†	3.36† *ddd*‡	3.30–3.43 *m*	4.97 5.05 *m*	3.32–3.36 *m*	4.65 *m*	5.30† *m*	4.55–4.46 *m*	4.95 *m*
H-4						—	5.30 *m*	—	
H-15	2.59 *m*	2.59 *m*	6.70 *d*§	6.72 *d*§	6.75 *d*§	6.71 *d*§	2.59 *m*	6.71 *d*§	6.71 *d*§
H-16	6.06 *t*‡	6.06 *t*‡	6.15 *d*§	6.15 *d*§	6.13 *d*§	6.13 *d*§	6.07 *t*‡	6.06 *d*§	6.15 *d*§
H-18	1.22 *s*	1.20 *s*	1.22 *s*	1.22 *s*	1.20 *s*	1.22 *s*	1.25 *s*	1.25 *s*	1.22 *s*
H-19	1.04 *s*	1.05 *s*	1.11 *s*	1.11 *s*	1.12 *s*	1.11 *s*	1.06 *s*	1.11 *s*	1.11 *s*
H-21a	5.00 *dd*‡	4.92 *dd*‡	5.05 *br s*	5.15 *br s*	5.05 *br s*	5.05 *br s*	4.90 *dd*‡	5.15 *br s*	5.05 *br s*
H-21b	4.80 *dd*‡	4.96 *dd*‡	5.05 *br s*	5.15 *br s*	5.05 *br s*	5.05 *br s*	4.97 *dd*‡	5.15 *br s*	5.05 *br s*
H-22	5.95 *dd*‡	5.95 *dd*‡	5.81 *br s*	5.95 *br s*	5.88 *br s*	5.81 *br s*	5.95 *dd*‡	5.88 *s*	5.81 *s*
OAc				2.04 *s*	—		—	2.06 *s*	2.05 *s*
				2.16 *s*		—		2.16 *s*	—
H-1′	4.56 *dd*‡	4.40 *dd*‡		—	—	—		—	
H-3′, H-4′, H-5′	3.37–3.75 *m*	3.46 *m*				—		—	—
H-6′	1.33 *d*‡	1.32 *d*‡			—		—		—
OMe	3.38 *s*	3.38 *s*	—		—				

•$J_{1,2}$ = 10.2 Hz.

†Assignments may be reversed.

‡Multiplicities: $J_{1a,2\beta} = J_{1\beta,2\beta} = J_{2\beta,3a} = J_{3a,4a} = J_{3a,4\beta}$ = 4.9 Hz; $J_{15a,16} = J_{15\beta,16}$ = 2.73 Hz; $J_{21a,21b}$ = 16 Hz; $J_{21a,22} = J_{21b,22}$ = 1.4 Hz; $J_{1a,2a}$ = 2.0 Hz; $J_{1a,2\beta}$ = 9.75 Hz; $J_{5,6}$ = 7.1 Hz.

§$J_{15,16}$ = 2.37 Hz.

Table 2. ^{13}C NMR chemical shifts of cardenolides 1 and 2 (75 MHz, $CDCl_3$)

C	1	2	C	1	2
1	45.8	45.6	16	133.8	132.1
2	72.4*	72.4*	17	161.0	157.6
3	72.8*	72.5*	18	15.7	16.2
4	29.7	29.7	19	24.5	24.8
5	36.3†	36.3†	20	172.8	173.5‡
6	26.8	26.9	21	71.4	72.4
7	19.9‡	19.9	22	113.0	116.9
8	65.1	36.8†	23	169.5	174.1‡
9	36.5†	36.5†	1'	99.0	98.05
10	33.5	33.4	2'	32.1	32.1
11	20.1‡	24.6	3'	78.15	78.1
12	40.0	37.0	4'	67.2	67.2
13	49.5	51.4	5'	70.4	70.4
14	70.5	85.8	6'	16.8	16.8
15	38.0	37.0	OMe	55.7	55.7

All values are in (ppm) relative to TMS.
*,†,‡Assignments may be reversed.

that the sugar moiety in 2 was also D-diginose. The data of 2 showed its close analogy with 1, while the molecular formula indicated that instead of the epoxy ring, 2 had a hydroxyl function which could be located at C-14 in the light of the following observations.

Compound 2, on hydrolysis, afforded diginose identified by paper chromatography, together with three components which were characterized as 5, 6 and 7 on the basis of chemical and spectral data. Compound 5 had molecular formula $C_{23}H_{30}O_4$ (high-resolution mass spectrometry), mp 98–99° and an UV maximum at 337 nm. The ^1H NMR spectrum showed two doublets at δ6.75 (J = 2.37 Hz, H-15) and 6.13 (J = 2.37 Hz, H-16), a broad singlet at 5.88 (H-22) and signals of the carbinylic protons between 3.32 and 3.36. On acetylation, 5 formed the diacetyl derivative (8) with molecular formula $C_{27}H_{34}O_6$, showing two sharp singlets at δ2.16 and 2.06 for the acetoxymethyl protons in ^1H NMR spectrum. All these data are in agreement with the structure assigned to 5.

The molecular formula of 6 ($C_{23}H_{28}O_3$, high-resolution mass spectrometry) and the UV and ^1H NMR spectral data showed that 6 had the same polyene system as that observed in 5, but the hydroxyl function at C-2 had also been eliminated during hydrolysis. Thus the ^1H NMR spectrum (Table 1) showed a one-proton doublet at δ5.29 (J = 10.28 Hz) and a multiplet at δ5.34 for H-1 and H-2, respectively. This was confirmed by the acetylation of 6, which gave the monoacetyl derivative (9), with molecular formula $C_{25}H_{30}O_4$, and a sharp singlet at δ2.05 in the ^1H NMR spectrum for the acetoxymethyl protons.

Compound 7 had the same molecular formula, $C_{23}H_{30}O_4$ (high-resolution mass spectrometry) as determined for 5, but its UV maximum at 267 nm and the presence of a triplet at δ6.07 ($J_{15\alpha,16} = J_{15\beta,16}$ = 2.73 Hz, H-16) in the ^1H NMR spectrum (Table 1) showed that it had only one double bond in ring D, i.e. at C-16. The other double bond could be placed at C-3, since the ^1H NMR spectrum showed two multiplets at δ4.71 (H-2) and 5.30 (H-3 and H-4). The signals for H-21 and H-22 were also observed, as noted in Table 1. The β-configuration of the glycosidic linkage was deduced from the coupling constant of the anomeric proton, and the structure 2 was finally assigned to neriumoside which was substantiated by the fragments observed in the mass spectrum (see Experimental) and by the ^{13}C NMR spectral data (Table 2).

EXPERIMENTAL

Mps were recorded in glass capillary tubes and are uncorr. ^1H NMR and ^{13}C NMR (broad band and DEPT) spectra were recorded in $CDCl_3$ on Bruker WP-100 SY FT-NMR and AM-300 MHz spectrometers with TMS as internal reference. ^{13}C NMR spectral assignments were made partly through comparison of the chemical shifts with the data published for similar compounds [8, 9] and partly through the appearance of signals in the DEPT spectrum. The purity of the samples was checked on TLC (silica gel SIF-254 precoated Al sheets). Paper chromatograms for sugars were run in the solvent system toluene–BuOH (1:9) saturated with H_2O [10]. Leaves of *N. oleander* were identified by Dr. Saeeda Qureshi, Department of Botany, University of Karachi. A voucher specimen (N.OL-1) has been deposited at the Herbarium of the Botany Department, University of Karachi.

The residue left on removal of the solvent from the combined methanolic percolates of the fresh, undried and uncrushed leaves of *N. oleander*, collected in October from the Karachi region, was divided into acidic, basic and neutral fractions. The neutral fraction was taken in 90% MeOH and successively shaken out with petrol and petrol–C_6H_6 (1:1). The residue obtained from the methanolic phase after usual work-up was dissolved in C_6H_6 and the soln treated with a little petrol. A small amount of insoluble darkish ppt. was filtered off and the filtrate freed of solvent under red. pres. The light yellow, powdery residue was then subjected to prep. TLC (silica gel; C_6H_6–EtOAC, 4:1) through which 1 and 2 were ultimately obtained as uniform constituents in 0.15 and 0.5% yield, respectively (based on the weight of the neutral fraction).

Kaneroside (1). Needles (MeOH C_6H_6, 1:1), mp 110–111°, $[\alpha]_D^{24}$ + 26.66° ($CHCl_3$); FAB MS m/z: 531.2952 $[MH]^+$ (calc. for $C_{30}H_{43}O_8$: 531.2957); EIMS m/z: 368.1994 $[C_{23}H_{28}O_4]^+$ (fragment a), 355.1926 $[C_{22}H_{27}O_4]^+$ (fragment b), 145.0882 $[C_9H_{13}O_3]^+$, (fragment c); UV λ_{max}^{MeOH} nm: 218, 267; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3450, 1780, 1750, 1625.

Hydrolysis of kaneroside. Kaneroside (50 mg) in 10 ml EtOH was heated with 10 ml 0.05 M HCl for 5 min. The reaction mixture was partitioned between EtOAc and H_2O, and the EtOAc layer on usual work-up afforded aglycone 3, which formed colourless needles on keeping its conc. $CHCl_3$ soln in the cold, mp 138–140°. EIMS m/z: 368.1972 $[M]^+$ ($C_{23}H_{28}O_4$ requires: 368.1987); UV λ_{max}^{MeOH} nm: 386; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3450, 1780, 1740. The sugar was identified as D-diginose by PC of the aq. layer: R_f = 0.72 [11].

Acetylation of compound 3. Acetylation of 3 with Ac_2O–C_5H_5N at room temp. overnight afforded the diacetate 4; rods (EtOAc); mp 180–181°; EIMS m/z: 452.2100 $[M]^+$ ($C_{27}H_{32}O_6$ requires: 452.2198). UV λ_{max}^{MeOH} nm: 385; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1780, 1740 (br), 1640.

Neriumoside (2). Needles (MeOH–C_6H_6, 1:1), mp 140–142°; $[\alpha]_D^{24}$ + 23.20° ($CHCl_3$). FAB MS m/z: 533.3109 $[MH]^+$, (calc. for $C_{30}H_{45}O_8$: 533.3114); EIMS m/z 370.2130 $[C_{23}H_{30}O_4]^+$ (fragment a), 355.222 $[C_{23}H_{30}O_4 - Me]^+$, 145.089 $[C_9H_{13}O_3]^+$; UV λ_{max}^{MeOH} nm: 218, 267; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3450, 1780, 1750, 1625.

Hydrolysis of neriumoside. Hydrolysis of 2 was carried out

following the procedure described for kaneroside. The aglycone fraction obtained on usual work-up, was subjected to prep. TLC, which gave three components, **5**, **6** and **7**. The sugar was identified as D-diginose by PC: $R_f = 0.72$ [11].

Physical constants of compound **5**. Needles ($CHCl_3$), mp 98–99°; EIMS *m/z*: 370.2127 $[M]^+$ ($C_{23}H_{30}O_4$ requires: 370.2143); UV λ_{max}^{MeOH} nm: 337; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3410, 1780 (sh), 1740, 1640.

Acetylation of compound **5**. Acetylation of **5** with Ac_2O–C_5H_5N at room temp. afforded the diacetate **8**, irregular plates (EtOAc), mp 148–149°; EIMS *m/z*: 454.2362 $[M]^+$ ($C_{27}H_{34}O_6$ requires: 454.2355); UV λ_{max}^{MeOH} nm: 337; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1780, 1740 (br), 1630.

Physical constants of compound **6**. Needles ($CHCl_3$), mp 129–130°; EIMS *m/z*: 352.2038 $[M]^+$ ($C_{23}H_{28}O_3$ requires: 352.2033); UV λ_{max}^{MeOH} nm: 337; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400, 1780 (sh), 1740, 1630, 1600.

Acetylation of compound **6**. Acetylation with Ac_2O–C_5H_5N afforded monoacetate **9**, irregular plates (EtOAc), mp 120–121°; EIMS *m/z*: 394.2131 $[M]^+$ ($C_{25}H_{30}O_4$ requires: 394.2143); UV λ_{max}^{MeOH} nm: 337; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1780 (sh), 1740 (br), 1630.

Physical constants of compound **7**. Rods ($CHCl_3$), mp 125–126°; EIMS *m/z*: 370.2138 $[M]^+$ ($C_{23}H_{30}O_4$ requires: 370.2143); UV λ_{max}^{MeOH} nm: 267; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400, 1780, 1740, 1620.

REFERENCES

1. Dymock, W. (1890) *Pharmacographia Indica*, Vol. 2, p. 398. The Institute of Health and Tibbi Research. Republished under the auspices of Hamdard National Foundation of Pakistan.
2. Chopra, R. N., Nayar, S. L. and Chopra, I. C. (1956) *Glossary of Indian Medicinal Plants*, p. 175. C.S.I.R., New Delhi.
3. Abe, F. and Yamauchi, T. (1979) *Chem. Pharm. Bull.*, **27**, 1604.
4. Fieser, L. F. and Fieser, M. (1959) *Steroids*, p. 734. Verlag Chemie, Weinheim.
5. Yamauchi, T., Yujiro, M. and Yasuko, O. (1973) *Phytochemistry* **12**, 2737.
6. Hariharon, H. and Rangaswami, S. (1970) *Phytochemistry* **9**, 409.
7. Hiroji, I., Yasushi, O. and Hideo, I. (1985) *Phytochemistry* **11**, 2655.
8. Tori, K. and Ishii, H. (1973) *Tetrahedron Letters* 1077.
9. Brown, L., Cheung, H. T. A., Thomas, R. and Watson, T. R. (1981) *J. Chem. Soc. Perkin Trans.* 1, 1779.
10. Renkonen, O. and Schindler, P. (1956) *Helv. Chim. Acta* **39**, 1490.
11. Yamauchi, T., Naoe, T. and Tomiko, M. (1975) *Phytochemistry* **14**, 1379.