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ALKALOIDS OF *BOLUSANTHUS SPECIOSUS*

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Key Word Index—*Bolusanthus speciosus*; Leguminosae; ten quinolizidine alkaloids; novel 6β-hydroxylupanine.

Abstract—Investigation of the alkaloids of *Bolusanthus speciosus* afforded a new quinolizidine alkaloid, 6β-hydroxylupanine. The structure of this alkaloid was assigned on the basis of spectroscopic methods and by chemical transformations. The other alkaloids isolated were cytisine, *N*-methylcytisine, 11α-allylcytisine, anagyrine, 13-hydroxyanagyrine, 5,6-dehydrolupanine, lupanine, sparteine and β-isoparteine. The biosynthetic significance of these findings is discussed briefly.

INTRODUCTION

Bolusanthus speciosus is a monotypic genus which is widely distributed in Southern Africa. The trees often grow up to a height of 30–40 ft and possess pea-shaped violet blue flowers from which they gain the name South African Wisteria [1]. To date, there appears to have been only one chemical study published on this plant, in which the isolation of two tricyclic alkaloids, cytisine and *N*-methylcytisine is described [2]. In this communication, a further eight alkaloids are reported from *B. speciosus*.

RESULTS AND DISCUSSION

Preparative TLC of the alkaloid extract obtained from the leaves of *B. speciosus* yielded 10 alkaloids. Nine of these alkaloids were identified as sparteine (**2**), β-isosparteine (**3**), lupanine (**4**), 5,6-dehydrolupanine (**6**), anagyrine (**7**), 13-hydroxyanagyrine (**8**), 11α-allylcytisine (**9**), cytisine (**10**) and N-methylcytisine (**11**) by comparison of ^{13}C NMR spectra (Table 1), other spectroscopic and chromatographic data reported in the literature [3–6] and in some instances by direct comparison with authentic samples. One of the ten isolated alkaloids did not exhibit spectroscopic or chromatographic properties of any known compound and it has been characterised as 6β-hydroxylupanine (**5**), an unstable alkaloid which readily dehydrates to 5,6-dehydrolupanine (**6**). Chromatographic investigation of the alkaloids obtained from the seeds and the stem bark indicated that the ten alkaloids (**2**–**11**) were present in the seeds and that eight of these alkaloids were present in the stem bark (**3** and **4** were not detected). Cytisine (**10**) was the major alkaloid of the leaves, seeds and stem bark. The yields of alkaloids obtained from leaves, stem bark and seeds are given in Table 1.

In establishing the structure of the novel 6β-hydroxylupanine (**5**) it was imperative to establish unequivocally the identity of its dehydration product as 5,6-dehydrolupanine (**6**). The UV spectrum of **6** exhibited a λ_{max} at 250 nm which is typical of a vinyl amide [7] and its EIMS has an $[M]^+$ at *m/z* 246 (two mu less than lupanine). Catalytic hydrogenation of **6** yielded lupanine (**4**) identified on the basis of its TLC, mass spectrum, ^{13}C and ^{1}H NMR properties. Hence alkaloid **6** was lupanine with a single double bond which must be situated at C-5/C-6, C-6/C-7 or C-9/C-10. The ^{1}H NMR spectrum of **6** showed the presence of a four line system at δ4.95 (^3J = 5.9 and ^3J = 3.9 Hz) which was attributed to an olefinic proton and the ^{13}C NMR spectrum (Table 2) showed the presence of one tertiary vinylic carbon. Therefore, the double bond could not be at C-6/C-7. The presence of the double bond at C-9/C-10 was also excluded since a C-10 olefinic proton would be expected to appear as either a singlet or a multiplet with only small long-range coupling constants. Furthermore, in the ^{1}H NMR spectrum of **6** there was a multiplet at δ4.05 which is typical of the C-10 equatorial proton [8]. Hence the structure of **6** was established as 5,6-dehydrolupanine and its relationship with the novel 6β-hydroxylupanine (**5**) became apparent. The FAB mass spectrum of **5** showed an $[M + H]^+$ ion at *m/z* 265 which was 18 mu higher than that of **6**, consistent with the presence of an additional hydroxyl substituent on **5**. The position of the hydroxyl group at C-6 was established on the basis of the following considerations. (a) The lack of a signal in the δ3.3–4.0 region of the ^{1}H NMR spectrum of **5** which would be expected for a proton of the C<u>H</u>–OH type [9–11]. (b) The lack of signals

Table 1. Yields of alkaloids of *Bolusanthus speciosus* from leaves, stem bark and seeds, calculated as mg per 100 g of dry plant material

Alkaloid	Leaves	Stem bark	Seeds
β-Isosparteine	2.0	—	4.0
Sparteine	8.2	13.0	25.0
13-Hydroxyanagyrine	2.0	1.0	3.0
Cytisine	68.0	41.0	142.0
11α-Allylcytisine	20.0	9.0	48.0
N-Methylcytisine	28.0	14.0	64.0
Anagyrine	32.0	24.0	71.0
Lupanine	2.3	—	6.0
5,6-Dehydrolupanine	0.9	7.1	51.0
6β-Hydroxylupanine	7.6	0.9	6.0

Table 2. ^{13}C NMR chemical shifts of alkaloids isolated from *Bolusanthus speciosus**

C	2†	4†	5	6	7†	9	10†	11
2	55.9	171.5	172.0	171.2	163.6	163.6	163.6	163.9
3	25.6	33.0	33.2	31.8	116.8	116.9	116.5	116.9
4	24.6	19.6	19.2	19.2	138.9	138.9	138.5	139.0
5	29.1	26.7	32.2	103.5	104.8	105.1	104.6	105.1
6	66.2	60.9	85.5	142.2	151.9	150.9	151.4	151.4
7	35.8	34.8	37.5	33.4	35.4	35.0	35.3	35.6
8	27.3	27.4	15.9	24.4	22.9	21.1	25.3	26.4
9	32.8	32.2	34.3	33.0	32.6	30.0	27.8	27.8
10	61.7	46.8	42.5	47.5	51.4	47.4	49.9	49.8
11	64.1	64.1	64.3	63.4	63.1	58.0	62.1	53.0
12	34.3	33.8	34.2	28.2	25.4	—	—	—
13	24.2	24.4	24.1	21.6	19.2	51.2	—	54.1
14	25.6	25.2	24.1	22.8	20.8	34.8	46.1	—
15	55.1	55.5	55.3	56.6	53.1	135.4	—	—
16	—	—	—	—	—	117.6	—	—
17	53.2	52.9	54.1	54.9	54.4	—	—	—

*Spectra were determined in $CDCl_3$ solutions.

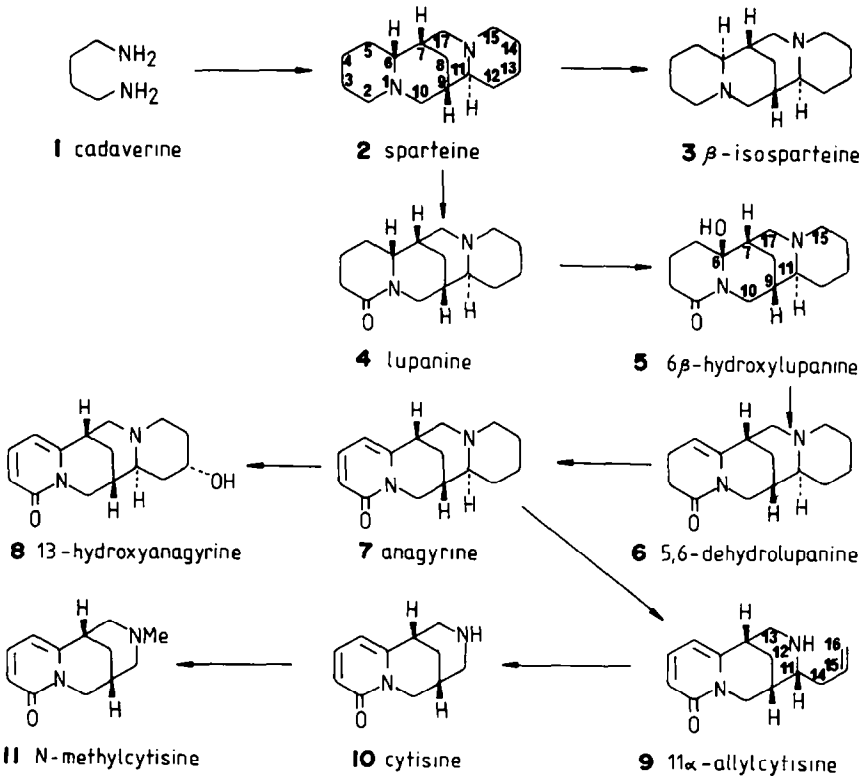
†^{13}C NMR chemical shifts have been reported previously [30] and the values given here are close to the published values.

in the δ3.0–3.4 region of the ^{1}H NMR spectrum of **5** where H-6 of a number of 2-oxosparteines has been reported to resonate [8, 9]. (c) The reduction of **5** with lithium aluminium hydride yielded sparteine (**2**) (identified by co-TLC and mass spectroscopy) which is consistent with the presence of a CO–N–C(–)–OH grouping in the molecule, and (d) the DEPT spectrum of **5** showed the presence of only three methine protons in contrast to lupanine (**4**) which possesses four. The ^{13}C signal for C-6 at 60.9 ppm in lupanine (**4**) was replaced in the spectrum of (**5**) by a quaternary carbon signal at 85.5 ppm (Table 2).

The establishment of the β-configuration of the hydroxyl substituent at **5** was made from CD considerations. The CD curve of **5** showed a Cotton Effect (CE) at 222 nm due to the inherently symmetrical lactam chromophore perturbed by the asymmetric environment. The CE was equal, but opposite in sign, to that of lupanine so that it was tempting to consider that the configuration of **5** at C-6 was opposite to that of lupanine (**4**). However, substituents such as hydroxyl groups are known to cause reversal of signs of rotation in CD spectra [12, 13]. The configuration of the C-6 hydroxyl of **5** was determined unequivocally from its benzoate derivative which showed a positive CE. Such positive exciton chirality (right handed screwness) is consistent with a β-configuration for the hydroxyl group of **5** [12].

The extraction methods employed influence the ratio of the yields of 6β-hydroxylupanine (**5**) to 5,6-dehydrolupanine (**6**). When cold methanol was used for initial extraction the ratio was 9:1, respectively, whereas when hot methanol was used this ratio was reversed. In the plant it is possible that **5** is stabilized in the cellular environment but during isolation procedures dehydration readily occurs. 5,6-Dehydrolupanine has been identified from various species of Leguminosae, mainly on the basis of GC/MS data [14–17], a technique which presupposes the use of high temperatures which would accelerate dehydration of **5** to **6**. Alkaloids containing bridgehead hydroxyl substituents appear to be rare in nature although two such matrine-type alkaloids have been reported from species of *Leontice* [18, 19], they are also known in the indolizidine series of alkaloids, e.g. 13a-hydroxysepticine [20] but they do not appear to have been reported from the pyrrolizidine series of alkaloids. It is highly possible that there are more bridgehead hydroxyl alkaloids present in nature than has previously been supposed and by using mild isolation techniques, it is to be anticipated that more 6-hydroxysparteine-type alkaloids will be discovered.

The biosynthesis of quinolizidine alkaloids has been studied extensively and it has been shown that the first committed step is the production of cadaverine (**1**) by decarboxylation of lysine [21–24]. Furthermore, it has been proposed that cadaverine is the precursor of a central intermediate, 17-oxosparteine [24, 25]. However, feeding of key labelled intermediates into *Lupinus angustifolius* and *L. luteus* has produced sufficient evidence to invalidate the role of 17-oxosparteine in the biosynthesis of lupanine and sparteine [26]. A plausible biosynthetic relationship between the 10 quinolizidine alkaloids present in *B. speciosus* is outlined in Scheme 1. Ring A of lupanine (**4**) would aromatize to give tetracyclic α-pyridone alkaloids of which anagyrine (**7**) is a representative [14, 17]. 5,6-Dehydrolupanine (**6**) is the likely intermediate between lupanine (**4**) and anagyrine (**7**), and **6** is present in species which contain both lupanine-type and α-pyridone-type alkaloids [14–17, 27, 28]. The unstable novel alkaloid 6β-hydroxylupanine (**5**) is the likely intermediate between lupanine (**4**) and 5,6-dehydrolupanine (**6**). Cleavage of the C-15/N-16 bond of anagyrine (**7**) would result in the formation of the tricyclic alkaloid 11α-allylcytisine (**9**) and subsequent loss of the C-11 allyl side chain would result in cytisine (**10**), the precursor of *N*-methylcytisine (**11**).



Scheme 1. Postulated biosynthetic pathway of quinolizidine alkaloids in *Bolusanthus* sp.

EXPERIMENTAL

IR spectra were recorded in $CHCl_3$ and UV spectra in MeOH. EIMS were obtained at 70 eV and FABMS were obtained using 8 K eV Ar with ion currents of 0.5 mA. Samples for FAB were prepared using glycerol as a matrix. ^1H and ^{13}C NMR spectra were obtained at 300 MHz and 62.5 MHz, respectively, and chemical shifts reported are relative to TMS. TLC on silica gel GF_{254} (Merck) utilized one of the following solvent systems: (A) $CHCl_3$–MeOH–28% NH_4OH (9:1); (B) *iso*-PrOH–EtOAc–$CHCl_3$–28% NH_4OH (11:4:4:1); (C) $CHCl_3$–Et_2NH (9:1); (D) Et_2O–MeOH–28% NH_4OH (44:5:1); (E) hexane–Et_2NH (7:3). Alkaloids were detected by spraying with Dragendorff's reagent.

Plant material. Bolusanthus speciosus (Bolus) Harms. leaves, seeds and stem bark were supplied by the Director of the Botanic Research Institute, Pretoria, S. Africa and a sample of leaves from the Director of the Lowveld Botanic Garden, Nelspruit, S. Africa.

Extraction and isolation of alkaloids. Powdered leaves from Pretoria (500 g) were macerated in MeOH for 48 hr. The filtered extract was concd to dryness under red. pres., extracted into 2% H_2SO_4 and washed with Et_2O (4 × 100 ml). The acidic aq. soln was made alkaline with 28% NH_4OH and extracted with $CHCl_3$ (3 × 100 ml). The combined $CHCl_3$ extracts were dried (Na_2SO_4) and evapd to dryness yielding a dark brown syrup (2.41 g, 0.48%). Leaves from Nelspruit (50 g), seeds from Pretoria (50 g) and stem bark from Pretoria (50 g) were extracted by the same procedure to yield 0.26 g (0.52%), 1.08 g (2.16%) and 0.14 g (0.28%), respectively. TLC of these four alkaloidal extracts indicated a similar alkaloidal composition to that from the extract from Pretoria leaves. Ten alkaloids were isolated from the extract of leaves from Pretoria using solvent systems A–E and by elution of the bands with $CHCl_3$–MeOH (4:1).

Sparteine (2). R_f 0.10 (system A, identical co-TLC with authentic sample); MS *m/z* (rel. int.): 234 $[M]^+$ (27), 193 (30), 137 (90), 136 (37), 110 (27), 98 (100), 97 (43).

β-Isosparteine (3). R_f 0.09 (system A); MS *m/z* (rel. int.): 234 $[M]^+$ (18), 193 (16), 137 (100), 136 (34), 110 (31), 98 (94), 97 (67) [29].

Lupanine (4). R_f 0.70 (system A), identical co-TLC with authentic sample; MS *m/z* (rel. int.): 248 $[M]^+$ (56), 219 (5), 150 (39), 149 (54), 148 (18), 136 (100), 112 (15), 110 (13), 98 (30), 97 (24); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2800–2700 (*trans*-quinolizidine, Bohlmann bands), 1622 (lactam CO).

6β-Hydroxylupanine (5). Yield 38 mg (0.01%); R_f 0.46 (system A); FABMS *m/z* (rel. int.): 265 $[M+H]^+$ (100), 248 (20), 148 (25), 136 (30), 134 (25), 98 (95); EIMS *m/z* (rel. int.) 264.1838 ($[M]^+$ 2; $C_{15}H_{24}N_2O_2$ calc. for 264.1839), 246 (39), 163 (5), 148 (7), 136 (8), 135 (6), 134 (8), 98 (100), 97 (29); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3300–3200 (OH), 2800–2700 (weak, Bohlmann bands), 1640 (lactam CO); ^1H NMR ($CDCl_3$): δ4.35 (H-10_{eq}, $J_{10eq,10ax}$ = 12.4 Hz, $J_{10eq,9}$ = 2.7 Hz), 3.10 (H-17_{eq}, $J_{17eq,17ax}$ = 11.4 Hz), 2.95 (H-10 ax, $J_{10ax,10eq}$ = 12.4 Hz, $J_{10ax,9}$ = 3.7 Hz), 2.80 (H-15_{eq}, $J_{15eq,15ax}$ = 11.8 Hz); CD (MeOH) Δ_{222} − 5.5; CD of 5 benzoate Δ_{233} + 7.8; CD lupanine (4) Δ_{222} + 5.5. *Dehydration.* 6β-Hydroxylupanine (5) (15 mg) was dissolved in $CHCl_3$ (3 ml), allowed to stand at room temp. for 12 hr to yield a single compound identified as 5,6-dehydrolupanine (6) (co-TLC, MS). *Benzoate.* 6β-Hydroxylupanine (15 mg) was dissolved in anhydrous pyridine (2 ml), Ph COCl (1 ml) was added and the mixture allowed to stand at room temp. overnight. The reaction mixture was evapd to dryness under red. pres. at 40°, 10% NH_4OH (3 ml) added and extracted with $CHCl_3$ (3 × 3 ml). The extract was then purified by prep. silica gel TLC using $CHCl_3$–MeOH–28% NH_4OH (95:5:1) to yield a yellowish oil (2.5 mg); MS *m/z* (rel. int.): 368 $[M]^+$ (21), 366 (18), 323 (11), 262 (100), 245 (45), 188

(14), 149 (32), 148 (14), 136 (32), 134 (36), 122 (45).

5,6-Dehydrolupanine (**6**). R_f 0.76 (system A); MS *m/z* (rel. int.): 246 $[M]^+$ (33), 163 (6), 148 (5), 136 (6), 135 (6), 134 (8), 98 (100), 97 (33); UV λ_{max}^{MeOH}: 250 nm; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2800–2900 (weak, Bohlmann bands), 1635 (lactam (CO); ^1H NMR ($CDCl_3$): δ4.95 (H-5, ^3J = 5.9 and ^3J = 3.5 Hz), 4.05 (H-10_{eq}, J = 13.1 and 1.8 Hz). *Hydrogenation.* 5,6-Dehydrolupanine (5 mg) was dissolved in EtOH (5 ml) and 5% Pd–C (1 mg) added. A stream of H_2 was passed through the soln for 15 min, the catalyst removed by filtration and the solvent evapd under N_2. The residue was dissolved in 2% H_2SO_4, filtered, adjusted to pH 9 with 28% NH_4OH and extracted with $CHCl_3$ (3 × 2 ml). The combined $CHCl_3$ extracts were washed, dried (Na_2SO_4), filtered and evapd to dryness under N_2 to yield a pale yellowish oil (3.2 mg) identified as lupanine (**4**) (co-TLC, MS).

Anagyrine (**7**). R_f 0.67 (system A); MS *m/z* (rel. int.): 244 $[M]^+$ (34), 146 (11), 136 (14), 98 (100), 161 (7), 160 (9); UV λ_{max}^{MeOH}: 233, 310 nm; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2800–2940 (*trans*-quinolizidine, Bohlmann bands), 1650, 1540 (α-pyridone).

13-Hydroxyanagyrine (**8**). R_f 0.37 (system A); MS *m/z* (rel. int.): 260 $[M]^+$ (27), 160 (13), 146 (27), 114 (100), 96 (40); UV λ_{max}^{MeOH}: 234, 311 nm; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3380 (OH), 2800–2920 (*trans*-quinolizidine, Bohlmann bands), 1650, 1540 (α-pyridone).

11 α-Allylcytisine (**9**). R_f 0.61 (system A); MS *m/z* (rel. int.): 230 $[M]^+$ (7), 202 (3), 189 (100), 160 (10), 148 (9), 147 (9), 148 (22), 134 (7); UV λ_{max}^{MeOH}: 234, 310 nm; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3340 (NH), 2750–2810 (*trans*-quinolizidine, Bohlmann bands), 1650, 1540 (α-pyridone).

Cytisine (**10**). R_f 0.40 (system A, identical co-TLC with an authentic sample); MS *m/z* (rel. int.): 190 $[M]^+$ (63), 160 (29), 148 (38), 147 (80), 146 (100), 134 (33), 118 (13), 117 (17), 109 (21); UV λ_{max}^{MeOH}: 233, 310 nm; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3350 (NH), 2940, 1650, 1550 (α-pyridone).

N-Methylcytisine (**11**). R_f 0.61 (system A, identical co TLC with an authentic sample); MS *m/z* (rel. int.): 204 $[M]^+$ (21), 189 (12), 160 (6), 146 (9), 58 (100); UV λ_{max}^{MeOH}: 234, 309 nm; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2940, 2780 (NMe), 1650, 1540 (α-pyridone).

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REFERENCES

1. De Winter, B., De Winter, M. and Killick, D. J. B. (1966) *Sixty Six Transvaal Trees*, p. 74. Transvaal Botanical Research Institute, Transvaal.
2. Smalberger, T. M., Vleggar, R. and De Wall, H. L. (1970) *Tydskr. Natuurwetensk* 10, 213 (*Chem. Abstr.* 1971, **74**, 95432).
3. Keller, W. J. and Hatfield, G. M. (1979) *Phytochemistry* **18**, 2068.
4. Cho, Y. D. and Martin, R. O. (1971) *Arch. Mass. Spec. Data* **2**, 328.
5. Faugeras, G., Paris, R. and Peltier, M. (1973) *Ann. Pharm. Franc.* **31**, 561.
6. Cho, Y. D. and Martin, R. O. (1972) *Arch. Mass. Spec. Data* **3**, 732.
7. Crow, W. D. (1959) *Aust. J. Chem.* **12**, 474.
8. Bohlmann, F., Schumann, D. and Schulz, H. (1965) *Tetrahedron Letters* 2705.
9. Daily, A., Dutschewska, H., Mollov, N. and Spassov, S. (1978) *Tetrahedron Letters* 1453.
10. Asres, K., Gibbons, W. A., Phillipson, J. D. and Mascagni, P., *J. Nat. Prod.* (in press).
11. Mascagni, P., Asres, K., Gibbons, W. A. and Phillipson, J. D., in preparation.
12. Klyne, W., Scopes, P. M., Thomas, R. N., Skolic, J., Grawrosnki, J. and Wiewiorowski, M. (1974) *J. Chem. Soc. Perkin Trans.* 1, 2565.
13. Bull, J. R. and Enslin, P. R. (1970) *Tetrahedron* **26**, 1525
14. Cho, Y. D. and Martin, R. O. (1971) *Can. J. Chem.* **49**, 265
15. Keller, W. J. and Zelenski, S. G. (1978) *J. Pharm. Sci.* **67**, 430.
16. Keller, W. J. and Ng, C. L. (1979) *Lloydia* **42**, 688.
17. Wink, M. and Hartmann, T. (1981) *J. Nat. Prod.* **44**, 14
18. Tkeshelashvili, E. G., Iskandarov, S., Mudzhin, K. S. and Yunusov, S. Yu. (1973) *Soobshch. Akad. Nauk. Gruz. S.S.R.* **69**, 357 (via *Chem. Abstr.* 1973, **78**, 156646).
19. Zunnunzhanov, A., Iskandarov, S. and Yunusov, S. Yu. (1974) *Khim. Prir. Soedin.* **10**, 115 (via *Chem. Abstr.* 1974, **80**, 121169).
20. Butani, K. K., Ali, M. and Atal, C. K. (1984) *Phytochemistry* **23**, 1765.
21. Mothes, K. and Schutte, H. R. (1969) *Biosynthese der Alkaloide*, p. 324. VEB, Berlin.
22. Nowacki, E. K. and Waller, G. R. (1977) *Rev. Latinoam. Quim.* **8**, 49.
23. Nowacki, E. K. and Waller, G. R. (1975) *Phytochemistry* **14**, 165.
24. Wink, M. and Hartmann, T. (1980) *Planta Med.* **40**, 149.
25. Hartmann, T., Schoofs, G. and Wink, M. (1980) *FEBS Letters* **115**, 35.
26. Golebiewski, W. M. and Spenser, I. D. (1984) *J. Am. Chem. Soc.* **106**, 7925.
27. Murakoshi, I., Kidoguchi, E., Ikram, M., Israr, M., Shafi, N., Haginawa, J., Ohmiya, S. and Otomasu, H. (1982) *Phytochemistry* **21**, 1313.
28. Murakoshi, I., Ito, I., Haginawa, J., Ohmiya, S., Otomasu, H. and Hirano, R. T. (1984) *Phytochemistry* **23**, 887.
29. Anderson, J. N. and Martin, R. O. (1976) *J. Org. Chem.* **41**, 3441.
30. Bohlmann, F. and Zeisberg, R. (1975) *Chem. Ber.* **108**, 1043.