Pergamon

Phytochemistry Vol. 49, No. 8, pp. 2579–2583, 1998

Printed in Great Britain
0031-9422/98/$ - see front matter

PII: S0031-9422(98)00429-4

ACETOPHENONES, A CHALCONE, A CHROMONE AND FLAVONOIDS FROM *PANCRATIUM MARITIMUM*

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(*Received* 18 *May* 1998)

Key Word Index—*Pancratium maritimum*; Amaryllidaceae; flowering bulbs; polyoxygenated acetophenones; 6,8-*di*-*C*-methylated flavonoids; chalcone; chromone; flavanone; chemotaxonomy.

Abstract—The ethanolic extract of fresh flowering bulbs of *Pancratium maritimum* L. yielded a new chromone, maritimin, two new polyoxygenated acetophenones, together with the flavonoids syzalterin, (−)-farrerol and (−)-liquiritigenin, and the chalcone isoliquiritigenin. The structures of the isolated compounds were determined through spectral analyses including NMR and MS studies. © 1998 Published by Elsevier Science Ltd. All rights reserved

INTRODUCTION

The genus *Pancratium* (family Amaryllidaceae, subfamily Amaryllidoidae) has attracted considerable attention due to the complex structural types of its alkaloids. The non-nitrogenous constituents of the Amaryllidaceae plants have not attracted much attention from phytochemists. In the 1980s Ghosal *et al.* reported on the isolation of chromones, chromone-glucosides and glucosyloxy acetophenones from *Pancratium biflorum* ([1–3]). Previous work on the non-nitrogenous constituents of the bulbs of the Egyptian *P. maritimum* by us resulted in the isolation and identification of two flavans together with three chromones ([4]). The flavonoids have been found to possess a wide range of pharmacological activities ([5, 6]). Therefore, we are interested to continue our efforts to identify the other flavonoid and phenolic constituents of the plant. Extensive column chromatography of the non-alkaloidal fraction of the defatted acidic organic layer of the ethanolic extract of the fresh bulbs (see Section 3) resulted in the isolation and characterization of a new chromone, maritimin (**5**), two new polyoxygenated acetophenones (**6** and **7**) together with the rare 6,8-*di*-*C*-methylated flavonoids syzalterin (**1**) ([7]) and (−)-farrerol (**2**) (Birch, Pettit, Ryan, & Speake, 1960). The flavanone (−)-liquiritigenin (**3**) ([8, 9]) together with the chalone isoliquiritigenin (**4**) ([8, 10]) were also isolated and identified.

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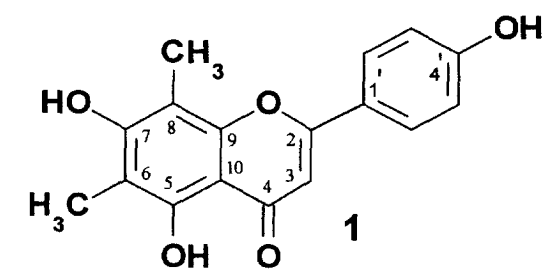
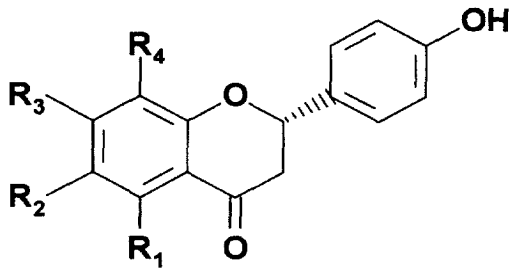
RESULTS AND DISCUSSION

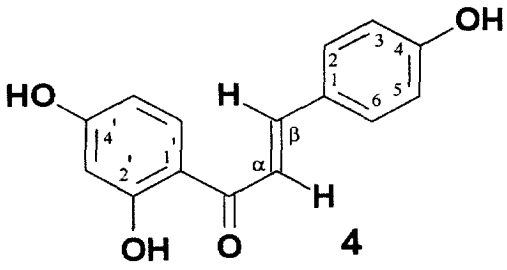
Syzalterin (**1**) was recently isolated from the leaves of *Syzygium afternifolium* ([7]). Its ^{1}H NMR spectrum showed resonances at δ 13.12 (1H), 6.77 (1H), 2.28 (3H), 2.02 (3H), 7.93 (2H) and 6.94 (2H) for OH-5, H-3, Me-8, Me-6, H-2′/H-6′ and H-3′/H-5′, respectively, and were in agreement with Ref. [7]. The ^{13}C NMR spectrum of **1** showed resonances for 17 carbons. The APT and DEPT experiments revealed the presence of 2 quartets (Me-6 and Me-8), 3 doublets (C-3, C-2′/C-6′ and C-3′/C-5′) and 10 singlets, among which were 5 oxygenated carbons which appeared downfield (δ 163.2 to 152.3) and corresponded to C-2, C-5, C-7, C-9 and C-4′, together with the resonances at δ 182.0, 103.4, 101.8, 106.9 and 121.5 for C-4, C-6, C-8, C-10 and C-1′, respectively. This is the first report for the ^{13}C NMR data of syzalterin (**1**).

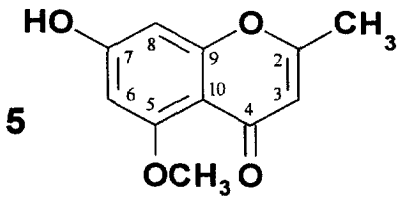
Comparison of the ^{1}H NMR data of **2** with those of **1**, revealed that compound **2** was the 2,3-dihydro derivative of **1**, based on the appearance of new signals at δ 5.46 (dd, $J_{2ax,3ax} = 12.3$ Hz, $J_{2ax,3eq} = 3.2$ Hz), 3.20 (dd, $J_{3ax,3eq} = 17.0$ Hz) and 2.71 (dd) for the protons H-2, H-3$_{eq}$ and H-3$_{ax}$, respectively, and the loss of the resonance of the olefinic H-3. The other resonances in the spectrum was comparable with those of **1**. The ^{13}C NMR and

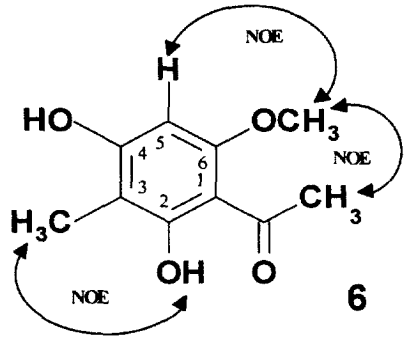


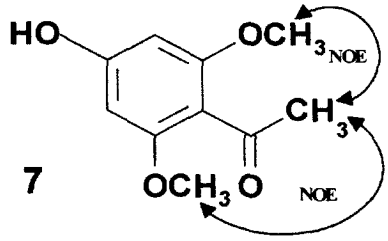


	R_1	R_2	R_3	R_4
2	OH	CH_3	OH	CH_3
3	H	H	OH	H









DEPT spectral data of **2** supported this finding through the appearance of a doublet at δ 78.0 for C-2 and a triplet at δ 42.0 for C-3 and the absence of the olefinic resonance of C-3. The remaining ^{13}C resonances were comparable with those of **1**. To the best of our knowledge, this is the first report of the ^{13}C NMR data of (−)-farrerol (**2**).

Both liquiritigenin (**3**) and isoliquiritigenin (**4**) are considered to be characteristic flavonoidal constituents of the Leguminosae family ([8]). The identification of (−)-liquiritigenin (**3**) was based mainly on the comparison of its spectral data ($[\alpha]_D$ and ^1H NMR) with those reported in [9]. Its ^{13}C NMR spectrum showed resonances for 15 carbons. These signals, based on APT and DEPT experiments, were classified into 6 doublets (C-2, C-5, C-6, C-8, C-2′/C-6′ and C-3′/C-5′), one triplet for C-3 and 6 singlets (C-4, C-7, C-9, C-10, C-1′ and C-4′). Among the singlets were three oxygenated ones appeared at δ 166.9, 165.3 and 156.8 for C-7, C-9 and C-4′, respectively. Other singlets were due to C-4, C-10 and C-1′.

The identfication of isoliquiritigenin **4** was based on the comparison of its spectral data (m.p. and ^1H NMR) with those reported in reference ([10]) and were in agreement with the published data ([10]).

The name maritimin was proposed for the new chromone **5**. Its HRMS showed a $[M]^+$ peak at *m/z* 206.0579 consistent with $C_{11}H_{10}O_4$. The IR spectrum showed characteristic bands at 3400 and 1660 cm^{-1} for OH and benz-pyrone, respectively. Its ^1H NMR spectrum showed signals due to two aromatic *m*-split doublets at δ 6.35 and 6.33 with J = 2.3 Hz, for H-8 and H-6, respectively, one *three*-proton singlet at δ 3.81 for an aromatic methoxy group and a C-Me group at δ 2.37 coupled with an olefinic proton at δ 5.98 (H-3, $^4J_{3,Me}$ = 0.8 Hz). Saturation of the signal of the C-2-Me caused collapse of the H-3 resonance into a sharp singlet. The diagnostic signal in the lower field (10–15 ppm) due to a chelated 5-hydroxyl group was not observed, showing that the methoxy group was at C-5 and the hydroxyl function at C-7, respectively. To the best of our knowledge, compound **5** has not been encountered before in nature nor has it been prepared synthetically.

Compound **6** was assigned the molecular formula $C_{10}H_{12}O_4$ based on the $[M]^+$ peak at *m/z* 196.0798 in the HRMS. Its IR spectrum showed absorptions

at 3220 and 1640 cm^{-1} for both chelated OH and CO groups, respectively. Its ^1H NMR spectrum showed a singlet at δ 14.21 for the strongly chelated proton of the OH at C-2, together with four singlets at δ 6.05 (1H), 3.79 (3H), 1.87 (3H) and 2.52 (3H) for H-5, OMe-6, Me-3 and Me-CO, respectively. The unequivocal determination of the structure of **6** was made only possible through NOE studies. For example, the position of the methyl group at C-3 was deduced from the NOE between the methyl singlet and the singlet of the chelated OH at δ 14.21, similarly the NOEs between the singlets resonating at δ 6.05 and 3.79 and between the singlets resonating at δ 3.79 and 2.52 suggested the position of the methoxyl group at C-6. The ^{13}C NMR spectrum of **6** revealed resonances for 10 carbons. Both APT and DEPT experiments showed three quartets at δ 7.2, 55.4 and 32.4 for Me-3, OMe-6 and Me-CO, respectively, one doublet at δ 90.3 for C-5 and 6 singlets, among which were three oxygenated carbons resonating at δ 163.9, 162.7 and 160.7 for C-2, C-4 and C-6, respectively. The other singlets appeared at δ 202.9 and 102.5 for CO and the overlapped resonances of C-1 and C-3, respectively. Compound **6** has not been previously reported as a natural metabolite nor synthesized.

Compound **7** was assigned the molecular formula of $C_{10}H_{12}O_4$ based on its EIMS and NMR spectral data. The IR spectrum displayed absorptions at 3230 and 1670 cm^{-1} due to OH and CO groups, respectively. Its ^1H NMR spectrum revealed signals due to a 2,4,6-trisubstituted acetophenone, based on the appearance of three singlets resonating at δ 3.65 (6H), 6.11 (2H) and 2.23 (3H) for the two methoxy groups at C-2 and C-6, the H-3 and H-5 and the methyl group of the acetophenone, respectively. The position of the methoxy groups at C-2 and C-6 was also confirmed by the NOE between the signals of the methyl group at δ 2.23 and that of the methoxyl groups at δ 3.65. The ^{13}C NMR and DEPT spectra revealed resonances for one doublet at δ 91.9 for C-3/C-5, two quartets at δ 55.4 and 32.4 for OMe-2/OMe-6 and CH_3 and finally two singlets at δ 160.1 and 157.6 for the oxygenated carbons C-4 and C-2/C-6, respectively, together with another singlet at δ 200.0 for CO. To the best of our knowledge, compound **7** was reported before as an enzymatic hydrolytic product of the acetophenone glucoside (2,6-dimethoxyacetophenone-4-O-β-D-glucoside) and identified through its UV and MS data ([3]), but this is the first report of its occurrence as a natural aglucone metabolite.

The co-occurrence of the rare 6,8-*di*-*C*-methylated flavonoid farrerol (**2**) together with syzalterin (**1**) in the bulbs of *P. maritimum* is noteworthy in the view of chemotaxonomy of genus *Pancratium* and in the biogenesis of 6,8-*di*-*C*-methylated polyoxygenated flavonoids.

EXPERIMENTAL

General

M.p.'s: uncorr; NMR: 400 MHz (for ^1H NMR) and at 100 MHz (for ^{13}C NMR); MS: Finnigan MAT-312 at 70 eV; MPLC: LiChroprep® SiO_2 and RP-18 (40–63 μm, Merck); TLC: pre-coated silica gel 60 F_{254} and RP-18 F_{254S} (0.25 mm, Merck). Spots were detected under UV (365 nm) and by spraying with 1% ethanolic $FeCl_3$ soln.

Plant material

P. maritimum bulbs were collected from plants cultivated at the campus of the Suez Canal University at Ismailia during the flowering period in July 1997. Samples were identified and authenticated by Professor Dr. N. El-Hadidy, Professor of Taxonomy, Cairo University and a voucher specimen has been deposited at the Herbarium of the Faculty of Pharmacy, Suez Canal University.

Extraction and isolation of **1–7**

Freshly collected bulbs (4.5 kg) were crushed and extracted by maceration in EtOH for 72 h (3 × 15 l). The combined extracts were evaporated and the concentrated viscous extract was partitioned between $CHCl_3$ and 2% H_2SO_4. The organic layer was concentrated under reduced pressure to give a viscous residue, F-1 (65 g). The acidic soln was made basic (pH 8–9) with Na_2CO_3 and extracted with $CHCl_3$. The combined organic layers were concentrated to give F-2 (9 g), which is currently under investigation. Fraction F-1 (65 g) was defatted by shaking with petrol. The defatted residue showed several $FeCl_3$-positive spots on TLC. The residue (45 g) was fractionated by flash CC eluting with petrol, followed by EtOAc–petrol gradients. Fractions of 50 ml were collected and monitored by TLC, Similar fractions were combined to give three main fractions (A–C). Fraction A showed $FeCl_3$-positive spots for compounds **5–7**, while compounds **1** and **2** contained in fraction B. Fraction C contained compounds **3** and **4**.

Fraction A (950 mg) was chromatographed on MPLC (LiChroprep SiO_2) using $CHCl_3$–MeOH (9:1) as an eluent. Fractions of 2 ml were collected. The residue of subfractions 23–27 (40 mg) gave on crystallization from MeOH 30 mg of **6**, while **7** (75 mg) was obtained on crystallization of the subfractions 31–38. The residue of subfractions 43–56 (35 mg) contained impure **5**, which was further purified on MPLC (LiChroprep SiO_2) using $CHCl_3$–MeOH (9:1), furnishing 7 mg of **5**. Fraction B (1300 mg) was subjected to CC on silica gel using $CHCl_3$–MeOH (9:1) as an eluent. Fractions of 10 ml were collected. Compound **1** (55 mg) was obtained as yellow needles upon crystallization from the methanolic soln of the subfractions 55–67 (95 mg) of this column. Repeated chromatography

of the subfractions 73–85 (120 mg) on MPLC (LiChroprep SiO_2) using $CHCl_3$–Me_2CO (4:1) as an eluent yielded **2** (65 mg). Fraction C (360 mg) was subjected to MPLC (LiChroprep RP-18) using MeOH–H_2O (2:1) as an eluent. Fractions of 2 ml were collected. The residue of the subfractions 7–9 (15 mg) gave upon crystallization 7 mg of **3**, while **4** (9 mg) was obtained on the rechromatography of the residue of the subfractions 13–18 (35 mg) on MPLC (LiChroprep RP-18) using MeOH–H_2O (2:1) as an eluent.

*Syzalterin (6,8-dimethyl-5,7,4-trihydroxyflavone) (**1**)*

Yellow needles (MeOH); $C_{17}H_{14}O_5$; m.p. 220–225° (dec.); IR and ^1H NMR (400 MHz, *DMSO-d_6*); were in agreement with [7]; ^{13}C NMR (100 MHz, *DMSO-d_6*); δ 182.0 (s, C-4), 163.2 (s, C-7), 160.9 (s, C-4'), 159.7 (s, C-2), 155.9 (s, C-5), 152.3 (s, C-9), 128.2 (d, C-2'/C-6'), 121.5 (s, C-1'), 115.9 (d, C-3'/C-5'), 106.9 (s, C-10), 103.4 (s, C-6), 102.4 (d, C-3), 101.8 (s, C-8), 8.2 (q, CH_3-6), 7.9 (q, CH_3-8); EIMS *m/z* (rel. int): 298 $[M]^+$ (100), 270 $[M-C{\equiv}O]^+$, (3), 180 $[C_9H_8O_4]^-$ (3), 152 $[180-C{\equiv}O]^+$ (11).

*(−)-Farrerol (6,8-dimethyl-5,7,4'-trihydroxyflavanone) (**2**)*

Pale yellow needles (MeOH); $C_{17}H_{16}O_5$; m.p. 213–215°; $[\alpha]_D^{25} = -23°$ (MeOH, *c* 0.1) (Lit. ([11]) = −20°, EtOH); IR ν_{max}^{KBr} cm^{-1}: 3320, 1656, 1600, 1460, 1380, 1330, 1180, 1030, 980, 830; ^1H NMR (400 MHz, *DMSO-d_6*); δ 12.37 (1H, s, exchangeable with D_2O, O*H*-5), 9.54 (1H, s, exchangeable with D_2O, O*H*-7), 7.30 (2H, m, *AA'*BB', H-2'/H-6'), 6.78 (2H, m, AA'*BB'*, H-3'/H-5') 5.46 (1H, dd, $J_{2ax,3ax} = 12.3$ Hz, $J_{2ax,3eq} = 3.2$ Hz, H-2_{ax}), 3.20 (1H, dd, $J_{3ax,3eq} = 17.0$ Hz, H-3_{ax}), 2.71 (1H, dd, H-3_{eq}), 1.95 (3H, s, CH_3-8), 1.93 (3H, s, CH_3-6); ^{13}C NMR (100 MHz, *DMSO-d_6*); δ 196.9 (s, C-4), 162.4 (s, C-7), 158.4 (s, C-5), 157.5 (s, C-4')*, 157.4 (s, C-9)*, 129.3 (s, C-1') 127.9 (d, C-2'/C-6') 115.2 (d, C-3'/C-5'), 103.2 (s, C-10), 102.5 (s, C-6), 101.8 (s, C-8), 78.0 (d, C-2), 42.0 (t, C-3), 8.2 (q, CH_3-6), 7.6 (q, CH_3-8); EIMS *m/z* (rel. int): 300 $[M]^+$ (74), 282 $[M-H_2O]^+$ (4), 180 $[C_9H_8O_4]^+$ (76), 152 $[180-C{\equiv}O]^+$ (100), 120 (15).

*(−)-Liquiritigenin (7,4'-dihydroxyflavanone) (**3**)*

White needles (MeOH); $C_{15}H_{12}O_4$; m.p. 205–207°; $[\alpha]_D^{25} = -36.2°$ (MeOH, *c* 0.09) (Lit. ([9]) = −34.5°, MeOH); ^1H NMR (400 MHz, *methanol-d_4*): were in agreement with [9]); ^{13}C NMR (100 MHz, *methanol-d_4*); δ 193.5 (s, C-4), 166.9 (s, C-7), 165.3 (s, C-9), 156.8 (s, C-4'), 131.3 (s, C-1') 129.8 (d, C-5), 129.0 (d, C-2', C-6'), 116.3 (d, C-3', C-5'), 114.9 (s, C-10), 111.7 (d, C-6), 103.8 (d, C-8), 81.0 (d, C-2), 44.9 (t, C-3).

*Isoliquiritigenin (4,2',4'-trihydroxychalcone) (**4**)*

Yellow needles (MeOH); $C_{15}H_{12}O_4$; m.p. 194–196°; ^1H NMR (400 MHz, *methanol-d_4*): were in agreement with [10]).

*Maritimin (7-hydroxy-5-methoxy-2-methylchromone) (**5**)*

Colorless prisms (Me_2CO); $C_{11}H_{10}O_4$; m.p. 112–113°; IR ν_{max}^{KBr} cm^{-1}: 3440, 1665, 1615, 1610, 1000, 995; ^1H NMR (400 MHz, *$CDCl_3$*) δ 6.35 (1H, d, $J_{6,8} = 2.3$ Hz, H-8), 6.33 (1H, d, $J_{6,8} = 2.3$ Hz, H-6), 5.98 (1H, d, $J_{3,Me} = 0.8$ Hz, H-3), 3.81 (3H, s, OCH_3-5), 2.37 (3H, brs, CH_3-2); HRMS: found 206.0579, calculated for $C_{11}H_{10}O_4$: 206.1976; EIMS *m/z* (rel. int): 206 $[M]^+$ (100), 178 $[M-C{\equiv}O]^+$ (11), 177 $[178-H]^+$ (94), 148 (21), 123 (18), 95 (22), 69 (39).

*2,4-Dihydroxy-6-methoxy-3-methylacetophenone (**6**)*

Pale yellow needles (MeOH); $C_{10}H_{12}O_4$; m.p. 202–203° (dec.); IR ν_{max}^{KBr} cm^{-1}: 3220, 1640, 1610, 1600, 1560, 1470, 1440, 1350, 1120, 800; ^1H NMR (400 MHz, *DMSO-d_6*); δ 14.21 (1H, s, exchangeable with D_2O, O*H*-2), 6.05 (1H, s, H-5), 3.79 (3H, s, OCH_3-6), 2.52 (3H, s, CH_3-3), 1.87 (3H, s, CH_3-CO); ^{13}C NMR (100 MHz, *DMSO-d_6*); δ 202.9 (s, *C*O), 163.9 (s, C-2), 162.7 (s, C-4), 160.7 (s, C-6), 102.5 (s, C-1 and C-3, overlapped signals), 90.3 (d, C-5), 55.4 (q, OCH_3-6), 32,4 (q, CH_3–CO), 7.2 (q, CH_3-3); HRMS: found 196.0798, calculated for $C_{10}H_{12}O_4$: 196.2012; EIMS *m/z* (rel. int): 196 $[M]^+$ (38), 181 $[M-CH_3]^+$ (100), 166 $[181-CH_3]^+$ (10), 138 $[166-C{\equiv}O]^+$ (5), 83 (5), 69 (7), 55 (7), 43 (15), 32 (17), 28 (72).

*2,6-Dimethoxy-4-hydroxyacetophenone (**7**)*

Pale yellow needles (Me_2CO) $C_{10}H_{12}O_4$; m.p. 76–78°; IR ν_{max}^{KBr} cm^{-1}: 3230, 1670, 1610, 1600, 1480, 1430, 1340, 1130, 1000, 820; ^1H NMR (400 MHz, *DMSO-d_6*); δ 6.07 (2H, s, H-3, H-5), 3.67 (6H, s, OCH_3-2/OCH_3-6), 2.29 (3H, s, CH_3–CO); ^{13}C NMR (100 MHz, *DMSO-d_6*); δ 200.0 (s, *C*O), 160.1 (s, C-4), 157.6 (s, C-2/C-6), 111.6 (s, C-1), 91.9 (d, C-3/C-5), 55.4 (q, OCH_3-2/OCH_3-6), 32.4 (q, CH_3); EIMS *m/z* (rel. int): 196 $[M]^+$ (15), 181 $[M-CH_3]^+$ (100), 166 $[181-CH_3]^+$ (15), 138 $[166-C{\equiv}O]^-$ (9), 123 (5), 95 (4), 69 (10), 43 (10), 28 (50).

Acknowledgements—Equipment from the World University Service, Wiesbaden, Germany, for the senior author's laboratory is gratefully acknowledged. We are grateful to Dr. W. Wörth, Institut für organische Chemie der Universität Freiburg, for recording the MS spectra. We would also like to thank Mr. F. Idelbi, Institut für analytische Chemie der Universität Freiburg, for measuring the NMR spectra.

REFERENCES

1. Ghosal, S., Singh, S. P., Bhagat, M. P. and Kumar, Y., *Phytochemistry*, 1982, **21,** 2943.
2. Ghosal, S., Kumar, Y., Singh, S. P. and Ahad, K., *Phytochemistry*, 1983, **22,** 2591.
3. Ghosal, S., Mittal, P., Kumar, Y. and Singh, S. K., *Phytochemistry*, 1989, **28,** 3193.
4. Ali, A. A., Makboul, M. A., Attia, A. A. and Ali, D. T., *Phytochemistry*, 1990, **29,** 627.
5. Spilková, J. and Hubik, J., *Pharmazie in unserer Zeit*, 1992, **21,** 174 and references cited therein.
6. Middleton, E., Jr.., *Pharmaceutical News*, 1994, **1,** 6.
7. Rao, J. R. and Rao, R. S., *Indian Journal of Chemistry*, 1991, **30B,** 66.
8. Wollenweber, E. and Dietz, V. H., *Phytochemistry*, 1981, **20,** 869.
9. Akiyama, K., Kawazu, K. and Kobayashi, A., *Zeitschrift für Naturforschung*, 1994, **49c,** 811.
10. Saitoh, T., Noguchi, H. and Shibata, S., *Chemical and Pharmaceutical Bulletin*, 1978, **26,** 144.
11. Birch, A. J., Pettit, D. G., Ryan, A. J. and Speake, R. N., *Journal of the Chemical Society*, 1960, 2063.