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A PHENOLIC CINNAMATE DIMER FROM *PSORALEA PLICATA*

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Key Word Index—*Psoralea plicata*; Leguminosae; sesquiterpenes; tocopherol; phenolic cinnamates; furanocoumarins; plicatin A and B; *p*-coumaric acid derivatives; phenolic dimers; steroids; triterpenes.

Abstract—Caryophyllene oxide, α-tocopherol, *Z* and *E*-werneria chromenes, two furanocoumarins, bakuchicin and psoralen, in addition to plicatin-B, lupeol and stigmasterol, have been isolated from the hexane-soluble extract of the aerial parts of *Psoralea plicata*. Plicatin-A, 3-(-3-methyl-2,3-epoxybutyl)-*p*-coumaric acid methyl ester and a new dimer, α-diplicatin B, were isolated from the ethyl acetate-soluble fraction. From the butanol-soluble matter, roseoside A, daidzin, isopsoralic acid-*O*-gluco-pyranosyl and isovitexin were isolated. Most of these compounds have been isolated from this species for the first time, although known from other plant sources. © 1997 Elsevier Science Ltd. All rights reserved

INTRODUCTION

The herb, *Psoralea plicata*, known in Arabic as 'Marmid' [1], is widely distributed in the Allaqi area, south of Aswan [2]. The plant is used by Bedouins as a grazing plant and for medicinal uses for different ailments.

Various species of *Psoralea* are reputed in indigenous medicine for their anthelmintic, antipyretic, analgesic, anti-inflammatory, diuretic and diaphoretic properties and are useful in bilious infections, in leprosy and for menstruation disorders [3, 4]. These reports prompted us to carry out systematic phytochemical studies in order to isolate the secondary metabolites found in these important medicinal plants. Previous work has identified the presence of furocoumarins, phenolic cinnamates, coumestans, terpenoids, isoflavones, flavonoids and α-tocopherolquinones [5–22].

RESULTS AND DISCUSSION

A methanol extract of the air-dried aerial parts of *P. plicata* was concentrated and the solvent-free residue exhaustively extracted with hexane, ethyl acetate and *n*-butanol, respectively. The residue obtained from each solvent was chromatographed as detailed in the Experimental section for isolation of its components.

Spectral analysis of compound **1** suggested that it should be β-caryophyllene oxide [23, 24]. The dimethyl protons appeared in the ^{1}H NMR at δ 0.99 and 1.00, and its ^{13}C NMR shifts at δ 21.5 and 29.8, respectively, in agreement with data reported for the *trans*-conformer of caryophyllene oxide [25, 26].

The second oily compound **2** was identified as α-tocopherol, in contrast to tocopherolquinone and its methyl ester reported from the same plant source [11, 27]. Our mass spectrum and fragmentation pattern agreed well with reported data for α-tocopherol [28].

Compounds **3** and **4** were identified as *Z*- and *E*-werneria chromenes, previously reported from *Werneria* species [29], but isolated from *P. plicata* for the first time. It should be noted that werneria chromene has been prepared semisynthetically by cyclization of plicatin-B, the anti-microbial agent isolated from several *Psoralea* species [10].

Compound **5** was bakuchicin, an angular furanocoumarin isolated previously from *P. corylifolia* [8]. Compounds **6** and **8** were identified as psoralen and plicatin-A, respectively, being common constituents in most *Psoralea* species [9, 10, 19].

The ^{1}H NMR spectrum of compound **7** showed two *ortho*-coupled aromatic protons at δ 7.30 and 6.80 (each 1H, *d*, $J = 8.89$ Hz) and an isolated proton singlet at δ 7.28. The presence of a *trans*-methyl cinnamate moiety was shown by a pair of doublets of olefinic protons at δ 6.28 and 7.62 ($J = 15.93$ Hz) and a sharp singlet for a methoxyl group at δ 3.78. In addition, the spectrum showed the presence of a pre-

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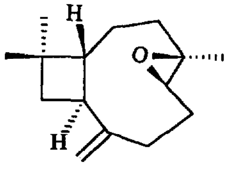

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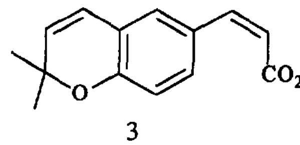

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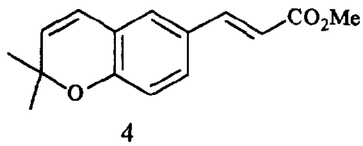

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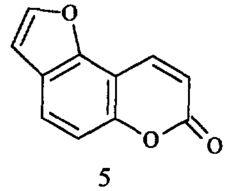

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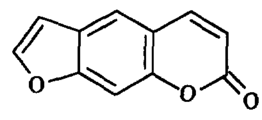

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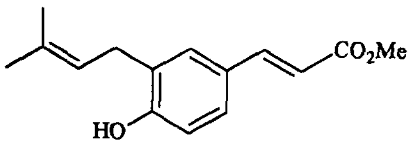

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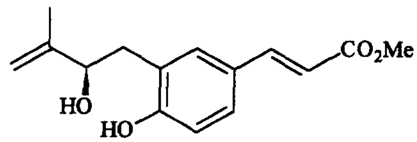

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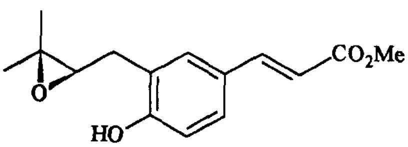

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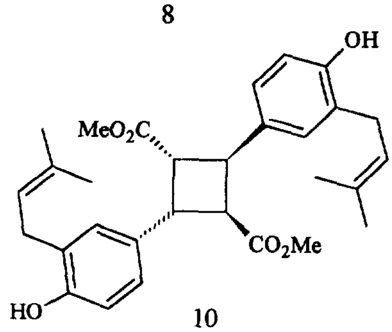

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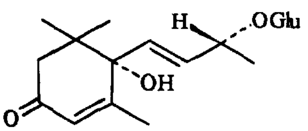

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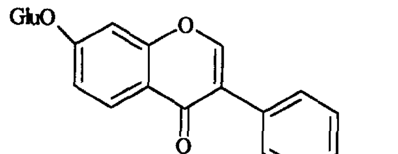

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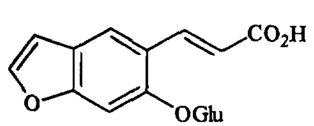

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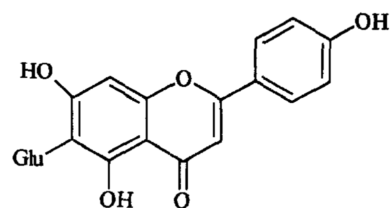

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nyl group (two methyl singlets at δ 1.76 and 1.78; 1H, multiplet at δ 5.31 and 2H, doublet at δ 3.35, J = 7.16 Hz). The EI mass spectrum of this compound showed a $[M]^+$ at m/z 246, giving the molecular formula $C_{15}H_{18}O_3$, with characteristic fragments at m/z 231 $[M-Me]^+$, 229 $[M-OH]^+$, 215 $[M-MeO]^+$ and 191 $[M-C_4H_7]^+$. The ^{13}C NMR spectrum determined in the DEPT mode, showed the presence of 15 carbon atoms with three methyls (δ 17.6, 25.6 and 51.6), one methylene (δ 28.5), six methines (δ 113.9, 115.7, 121.4, 127.5, 129.9 and 145.7), one carbonyl (δ 168.7) and four quaternary carbon atoms (δ 126.4, 128.0, 133.8

and 156.8). The set spectral data corresponded with plicatin B, an antibacterial compound isolated previously from *P. plicata* and *P. juncea* [9, 10].

Compound **9** was obtained as an oil and identified by spectral measurements as 3-(3-methyl-2-3-epoxy-butyl-)-*p*-coumaric acid methyl ester, an epoxide reported from *Baccaris* species [21]. It is the first time that this epoxide has been identified *Psoralea*.

The ^{1}H NMR spectrum of compound **10** showed three signals for aromatic protons, two doublets, at δ 6.65 and 6.99 (J = 8.9 Hz) and one broad singlet at δ 7.02. In addition, the spectrum showed the presence of a prenyl group (6H singlet at δ 1.88, 1H multiplet at δ 5.29 and 2H doublet at δ 3.20 (J = 7.1 Hz). Furthermore, the presence of two methine protons at δ 3.90 and 4.35, each being a double doublet (A_2B_2 pattern showing eight lines for H-1″ and H-2″, *J cis*/*J trans* 10.4/7.2) [30] and of the A_2B_2 pattern type was characteristic of cyclobutyl protons, since in this case each proton on the cyclobutane ring is *cis*-coupled with one of its neighbours and *trans*-coupled with the other; this suggested that compound **10** should be a dimer [31]. The ^{13}C NMR of compound **10** showed 15 carbon atoms, the same as that of plicatin-B, but compound **10** contained two methine carbons shifted upfield at δ 40.75 and 47.10; this is also characteristic of methine carbons in a cyclobutyl ring. In addition, there were four methine, one methylene, four quaternary carbons, one methoxyl, two methyls and one carbonyl carbon. The mass spectrum of compound **10** showed only peaks for the monomer but the corresponding spectrum of its diacetate showed a $[M]^+$ at m/z 576, giving the molecular formula $C_{34}H_{40}O_8$. This observation confirmed that compound **10** was a dimer of plicatin-B, given the name α-diplicatin B. Compound **10** is symmetrical, because only half of the total number of protons and carbon atoms are found in its ^{1}H and ^{13}C NMR spectra. Although the finding of a naturally occurring dimer, such as α-diplicatin B, is unusual, an equivalent dimer of cinnamic acid (referred to as gratissimic acid) together with its dimethyl ether (gratissimin) has been previously reported in *Ocimum gratissimum* [30], together with α-dicerptene in *Pityrogramma triangularis* [31].

From the *n*-butanol soluble extract, four glycosides, namely, roseoside A **11** [32–37], diadzin **12** [38–40], isopsoralic acid-*O*-glucopyranosyl **13** [41] and isovitexin **14** [42, 43] were isolated, respectively. The HC-correlations based on HMBC for the acetates of compounds **11** and **13** are presented in Figs 1 and 2.

The ^{1}H NMR, spectrum of compound **11**, showed a signal for one olefinic proton at δ 5.88 (1H, *br s*), which revealed in the correlation spectrum (HMBC), a cross-peak with the methyl group at δ 1.88 (3H, *d*, J = 1.8 Hz) on a quaternary carbon. The methylene protons at δ 2.43 and 2.25 (each 1H, *d*, J = 17.0 Hz, H-2α and β), showed cross-peaks with two geminal methyl groups at δ 1.01 and 1.07 (each, 3H, *s*). The UV spectrum revealed that compound **11** contained a conjugated chain, which was confirmed by ^{13}C NMR

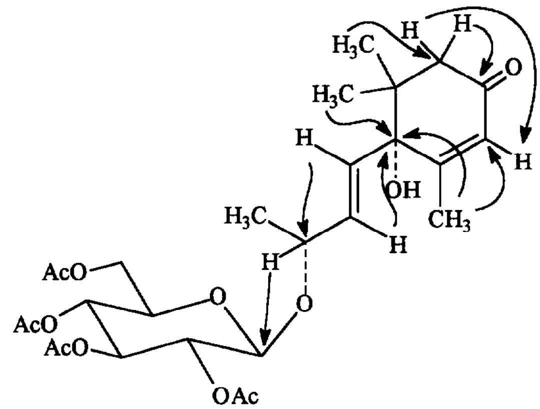

Fig. 1. ^{1}H-^{13}C correlation based on HMBC of roseoside A tetraacetate.

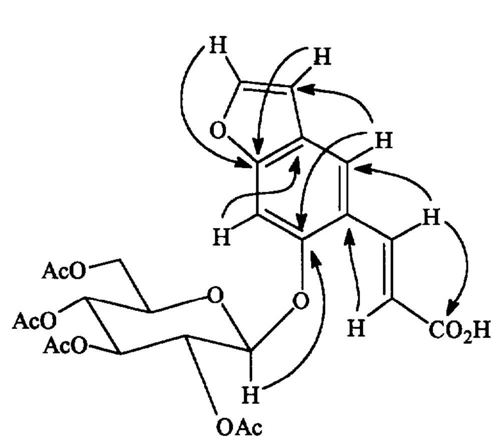

Fig. 2. ^{1}H-^{13}C correlation based on HMBC of isopsoralic acid-6-*O*-glucoside tetraacetate.

showing the characteristic shift for a ketonic carbon at δ 197.7. The ^{1}H NMR spectrum also showed two other olefinic protons at δ 5.77 (1H, *d*, J = 10.3 Hz) and δ 5.78 (1H, *dd*, J = 10.3 and 5.5 Hz), with the latter showing a cross-peak with a proton at δ 4.23 (1H, *m*), which, in turn, showed coupling (HH-COSY) with methyl protons at δ 1.23 (3H, *d*, J = 6.4 Hz). Compound **11** also showed the anomeric proton for glucose at δ 4.56 (1H, *d*, J = 7.9 Hz) which showed a cross-peak (CH-COSY) with the proton at δ 4.23, suggesting that the linkage of the sugar is at the OH on the carbon at δ 77.0. The ^{13}C NMR spectrum revealed the presence of one anomeric carbon at δ 100.0 and five other carbons (one methylene and four methines), besides four acetate group carbons. The coupling constant, NOESY and the chemical shift of the anomeric proton, confirmed that the sugar was β-linked. Acid hydrolysis of this compound with 5% HCl, gave D-glucose and vomifoliol (blumenol) [32–36]. From the above mentioned data and optical rotation, we found that compound **11** is similar to roseoside-A, previously isolated from *Vinca rosea* [37].

The ^{1}H NMR spectrum of compound **13** showed two furan protons at δ 7.60 (1H, *d*, J = 2.2 Hz) and δ 6.65 (1H, *dd*, J = 2.2 and 0.5 Hz). Also, there were two singlets for two aromatic protons at δ 7.79 and 7.37, in addition to a cinnamic acid group (two *trans*-coupled olefinic protons) at δ 8.04 and 6.94 ($J = 16.1$ Hz). The chemical shifts and splitting patterns of the furan, aromatic and olefinic protons were similar to psoralen, which was confirmed by a CH-COSY spectrum. The correlation spectrum (HMBC), showed a cross-peak between H-3 (δ 6.75), C-4 (δ 129.2) and C-8 (δ 156.5). Furthermore, H-2 (δ 7.60), displayed a cross-peak with C-9 (δ 123.6), that showed a cross-peak with H-7 (δ 7.37). From these correlations, we suggest that compound **13** has a benzofuran cinnamic acid structure. The ^{1}H NMR and ^{13}C NMR spectra showed one proton at δ 5.08 (1H, *d*, $J = 7.8$ Hz), its carbon at δ 100.3 showing a cross-peak with the carbon at δ 153.5, revealing that the sugar was β-linked to the carbon at δ 153.5. The ^{1}H NMR of compound **13** and ^{13}C NMR also showed four acetates and a sugar containing five methine carbons and one methylene group, indicating that the sugar was hexose. This was confirmed by acid hydrolysis with 5% HCl, giving glucose (TLC). From the above mentioned data and mass spectrum, we suggest that compound **13** was isopsoralic acid 1 → 6-*O*-β-D-glucoside, isolated previously from *Coronilla glauca* [41].

EXPERIMENTAL

^{1}H NMR and ^{13}C NMR (at 100 MHz), spectra were recorded using a Bruker AM-400 and Gemini-300, respectively, NOESY and HMBC on a Bruker AM-500.

Extraction and isolation of constituents. Air-dried aerial parts (2 kg) of *P. plicata* Del. (*Cullen plicatum* Delile C. H. Stirt) were powdered and exhaustively extracted with 75% MeOH by maceration. The alcohol extract was concd under red. pres. to a syrupy consistency (179 g). The solvent-free residue (50 g) was mixed with 200 ml H_2O and 100 ml MeOH, transferred to a separatory funnel and partitioned between hexane, EtOAc and *n*-BuOH. Each fr. was dried (Na_2SO_4) and concd to a syrupy residue (10 g hexane residue, 3.6 g EtOAc residue and 5 g *n*-BuOH residue).

Hexane-soluble fraction. Separation of compounds was achieved using repeated flash CC on silica gel, eluting with a hexane–EtOAc gradient. Compounds obtained were **1** (50 mg), **2** (25 mg), **3** (5 mg), **4** (30 mg), **5** (5 mg), **6** (18 mg), **7** (700 mg), lupeol (45 mg) and stigmasterol (10 mg).

Ethyl acetate-soluble fraction. The fr. (3.6 g) was slurried with 7 g of silica gel and transferred to a silica gel column, previously packed by the wet method in hexane–$CHCl_3$–MeOH (3.5:1.4:0.1), starting elution with the same solvent system to give pure epoxy compound **9** (7 mg) and the new dimer **10** (5 mg). The column was then eluted with MeOH and the MeOH eluate (1 g) subjected to ODS CC, eluting with MeOH–H_2O (4:1) to give another crop of compound **10** (30 mg).

Butanol-soluble fraction. The fr. (5 g) was dissolved in a small amount of MeOH, transferred to the top of a Sephadex-LH 20 column, previously packed in MeOH, and elution was started with MeOH (each fr. 250 ml) Each fr. obtained was conc. to 5 ml under red. pres. and analysed by TLC using EtOAc–MeOH–H_2O (100:16.5:13.5), where two subfrs, 1 and 2, were obtained.

Separation of compounds **11** *and* **12** *from subfr.* 1. Subfr. 1 (200 mg) was dissolved in a small amount of MeOH and transferred to a flash silica gel column previously packed with $CHCl_3$–MeOH–H_2O (30:20:1) and eluted by the same solvent to give two frs A and B.

Separation of compound **11** *by acetylation.* Fr A (20 mg) was dissolved in 2 ml pyridine and 1.9 ml Ac_2O and left for 24 hr at room temp. The reaction mixt. was tested by TLC for complete acetylation, then purified by flash silica gel CC eluted with hexane–Me_2CO (3:2), to give pure compound **11** acetate (17 mg).

Separation of compound **12** *by acetylation.* Fr. B (30 mg) was acetylated in a similar manner to fr. A and the mixt. purified by flash silica gel CC with toluene–MeOH (7:3), to give pure compound **12** acetate (20 mg).

Separation of compounds **13** *and* **14**. Subfr. 2 (500 mg), was dissolved in a small amount of MeOH and transferred to a flash silica gel column, previously packed with $CHCl_3$–MeOH–H_2O (37:12:1) and eluted with the same solvent system; 100 ml frs were collected Frs 15–25 gave compound **13** (30 mg) and frs 30–45 gave compound **14** (150 mg).

REFERENCES

1. Boulos, L., *Student's Flora of Egypt*. Al-Hadara Publishing, Dokki, Cairo, 1995.
2. Springuel, I., Allaqi Project Working Paper no. 13, 1994.
3. Steinmetz, E. F. and Lindermann, G., *Riechstaff, Aromen, Koerpepflegemittel*, 1964, **14**, 409.
4. Perry, L. M. and Metzger, J., *Medicinal Plants of East and South-East Asia, Attributed Properties and Uses*. The MIT Press, Cambridge, MA, 1980, p. 224.
5. Ahmed, V. U. and Basha, A., *Pakistan Journal of Science Industrial Research*, 1972, **15**, 158.
6. Zhu, D. Y., Chen, Z. X., Zhou, B. N., Liu, J. S., Huang, B. S., Xie, Y. Y. and Zeng, G. F., *Yao Hsueh Hasueh Pao*, 1979, **14**, 605.
7. Boardley, M., Stirton, C. H. and Harborne, J. B., *Biochemical Systematics and Ecology*, 1986, **14**, 603.
8. Kondo, Y., Kato, A., Kubota, Y. and Nozoe, S., *Heterocycles*, 1990, **31**, 187.
9. Rasool, N., Khan, A. Q. and Malik, A., *Phytochemistry*, 1990, **29**, 3979.
10. Schmitt, A., Talikepalli, H. and Mitscher, L. A., *Phytochemistry*, 1991, **30**, 3569.
11. Rasool, N., Khan, A. Q., Ahmed, V. U. and Malik, A., *Phytochemistry* , 1991, **30**, 2800.

12. Gupta, G. K., Dhar, K. L. and Atal, C. K., *Phytochemistry*, 1977, **16**, 403.
13. Gupta, B. K., Gupta, G. K., Dhar, K. L. and Atal, C. K., *Phytochemistry*, 1980, **19**, 2232.
14. Gupta, S., Jha, B. N., Gupta, G. K., Gupta, B. K. and Dhar, K. L., *Phytochemistry*, 1990, **29**, 2371.
15. Rasool, N., Khan, A. Q. and Malik, A., *Journal of Natural Products*, 1989, **52**, 749.
16. Mehta, G., Nayak, U. R. and Dev, S., *Tetrahedron*, 1973, **29**, 1119.
17. Wall, M. E., Wani, M. C., Manikumar, G., Abraham, P., Taylor, H., Hughes, T. J., Wamer, J. and Mcgiveney, R., *Journal of Natural Products*, 1988, **51**, 1084.
18. Suri, J. L., Gupta, G. K., Dhar, K. L. and Atal, C. K., *Phytochemistry*, 1978, **17**, 2046.
19. Lin, Y. L. and Kuo, Y. H., *Heterocycles*, 1992, **34**, 1555.
20. Gupta, G. K., Dhar, K. L. and Atal, C. K., *Phytochemistry*, 1978, **17**, 164.
21. Bohlmann, F., Knauf, W., King, R. M. and Robinson, H., *Phytochemistry*, 1979, **18**, 1011.
22. Montando, G., Caccamese, S. and Librando, V., *Organic Magnetic Resonance*, 1979, **6**, 534.
23. Warnhoff, E. W. and Srinivasan, V., *Canadian Journal of Chemistry*, 1973, **51**, 3955.
24. Maurer, B. and Grieder, A. C., *Helvetica Chimica Acta*, 1979, **60**, 2187.
25. Hinkley, S. F. R., Perry, N. B. and Weavers, R. T., *Phytochemistry*, 1994, **35**, 1479.
26. Thebtaranonth, C., Thebtaranonth, Y., Wanaupathamkul, S. and Yuthavong, Y., *Phytochemistry*, 1995, **40**, 125.
27. Teresa, J. P., Urones, J. G., Marcos, I. S., Ferreras, J. F., Bertelloni, A. M. and Barcala, P. B., *Phytochemistry*, 1987, **26**, 1481.
28. *Biochemical Applications of Mass Spectrometry*, ed. George R. Waller. Wiley, New York, 1972, p. 510.
29. Bohlmann, F., Zdero, C., King, R. M. and Robinson, H., *Phytochemistry*, 1984, **23**, 1135.
30. Vilian, C., Hubert, A., Dupont, L., Markam, K. R. and Walleniveber, E., *Zeitschrift für Naturforschung*, 1987, **24C**, 849.
31. Khuda, M., Ali, M. E., Khalique, A. and Shansuzzama, L. A., *Scientific Research*, 1964, **1**, 217.
32. Sholichin, M., Yamasaki, K., Kasai, R. and Tanaka, O., *Chemical and Pharmaceutical Bulletin*, 1980, **28**, 1006.
33. Thompson, M. J. and Dutky, S. R., *Phytochemistry*, 1972, **11**, 1748.
34. Pousset, J. L. and Poisson, J., *Tetrahedron Letters*, 1969, 1173.
35. Takasugi, M., Anetai, M., Katsui, N. and Masamune, T., *Chemical Letters*, 1973, 245.
36. Galbraith, M. N. and Hron, D. H. S., *Journal of the Chemical Society, Chemical Communications*, 1972, 113.
37. Bhakuni, D. S., Joshi, P. P., Uprety, H. and Kapil, R. S., *Phytochemistry*, 1974, **13**, 2541.
38. Kinjo, J. E., Furusawa, J. I., Baba, J., Takeshita, T., Yamasaki, M. and Nohara, T., *Chemical and Pharmaceutical Bulletin*, 1988, **35**, 4846.
39. Ohshima, Y., Okuyama, T., Takahashi, K., Takizawa, T. and Shibata, S., *Planta Medica*, 1988, 250.
40. Zapesochnaya, G. G. and Samylina, I. A., *Khimiya Priroduyku Soedinenii*, 1974, 671.
41. Stoll, A., Pereira, A. and Renz, J., *Helvetica Chimica Acta*, 1950, **33**, 1637.
42. Briggs, L. H. and Cambie, R. C., *Tetrahedron*, 1958, **2**, 269.
43. Tschesche, R. and Struckmeyer, K., *Chemisch Bevicht*, 1976, **109**, 2901.