Tetrahedron Vol. 39, No. 20, pp. 3351 to 3354, 1983
Printed in Great Britain.

0040-4020/83 $3.00 + .00
Pergamon Press Ltd.

PIMARANE AND CLEISTANTHANE DITERPENES FROM VELLOZIACEAE: ABSOLUTE CONFIGURATION AND BIOMIMETIC CONVERSION

A. C. PINTO*, M. L. PATITUCCI, R. S. DA SILVA, P. P. S. QUEIROZ and A. KELECOM
Núcleo de Pesquisas de Produtos Naturais, Universidade Federal do Rio de Janeiro, Centro de Ciências da Saúde, Bloco H, 21941, Cidade Universitária, Rio de Janeiro, RJ, Brazil

(*Received in the UK 26 November 1982*)

Abstract—Pimarane and cleistanthane diterpenes isolated from *Velloziaceae* are shown, by CD studies, to possess the same (5S, 10S) absolute configuration. A biomimetic rearrangement of the pimarane into the cleistanthane skeleton is described. The structure of a new natural diterpene, cleistantha-8, 11, 13-trien-7-one is also reported.

Pimarane diterpenes are well known secondary metabolites which have been found in several plant families.[1] In comparison, diterpenes with a cleistanthane skeleton are uncommon in Nature. The first members of this series were all isolated from species of the family Euphorbiaceae and are characterized by the presence of an aromatic C-ring (**1–4**).[2–4] Recently, a series of non-aromatic cleistanthanes related to structure **5** have been found in *Brickellia eupatoriedes* (Compositae).[5] Remarkably, all these diterpenes possess the (5R, 10R) absolute configuration as shown by CD studies on **5**[5] and on the cleistanthol keto-derivative **6**.[6]

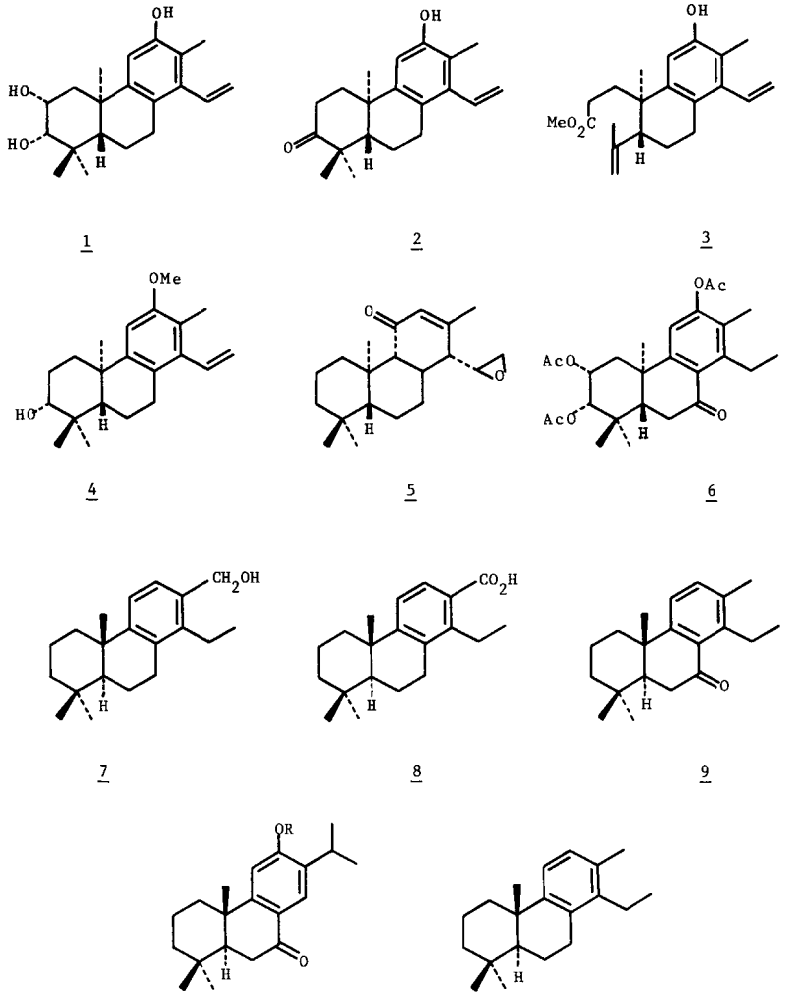
Some years ago, we reported[7] the isolation, from *Vellozia flavicans*, of veadeirol (**7**) and veadeiroic acid (**8**). On the course of our continuous studies on diterpenes from the family *Velloziaceae*, we have observed, in several species, the co-occurrence of pimarane[8] and cleistanthane derivatives.[9] Since primaranes are thought to be the biogenetic precursors of cleistanthanes,[6] it became of particular interest to determine the absolute configurations of the *Velloziaceae* diterpenses.

The key compound for determination of the absolute configuration in the cleistanthane series was isolated as a new natural product from *Vellozia leptopetala* and its structure identified to cleistantha-8, 11, 13-trien-7-one (**9**). It is a colourless crystalline compound (m.p. 89–90°; $[\alpha]_D = +35.5°$). The MS of **9** showed an intense molecular ion at *m/z* 284 compatible with the molecular formula $C_{20}H_{28}O$. The IR spectrum was characterized by a carbonyl absorption at 1675 cm^{-1} showing that the oxygen of **9** was part of an α, β-unsaturated ketone moiety. This was confirmed by the UV spectrum which exhibited absorptions at 222 (log $\epsilon = 3.79$), 257 (log $\epsilon = 3.84$) and 305 nm (log $\epsilon = 3.29$). The ^1H NMR spectrum indicated the presence of a 1, 2, 3, 4-tetrasubstituted aromatic ring, and the presence of five methyl groups, three of which on quaternary saturated carbons, one on the aromatic ring (2.30 ppm, 3H s) and the fifth being part of an ethyl group attached to the aromatic nucleus (1.21 ppm 3H t J = 7 Hz and 2.91 ppm 2H d J = 7 Hz). The ^1H NMR spectrum also showed, adjacent to the ketone, a methylene group (2.66 ppm 2H d J = 8 Hz), which, in turn, was adjacent to a methine (1H t J = 8 Hz at 1.77 ppm). Structure **9** was thus proposed for this new natural diterpene. ^{13}C NMR data were found consistent with proposed structure. The chemical shift of the angular methyl carbon (23.1 ppm) also proved the A/B ring junction to be *trans*.[7] The ORD and CD curves of **9** (see Experimental) showed a positive Cotton effect and a positive maximum similar to that of sugiol methyl-ether (**10a**)[10] but opposite in sign to that of cleistanthol derivative **6**.[6] Hence, cleistantha-8, 11, 13-trien-7-one (**9**) from *Vellozia leptopetala* had the (5S, 10S) absolute configuration.[11]

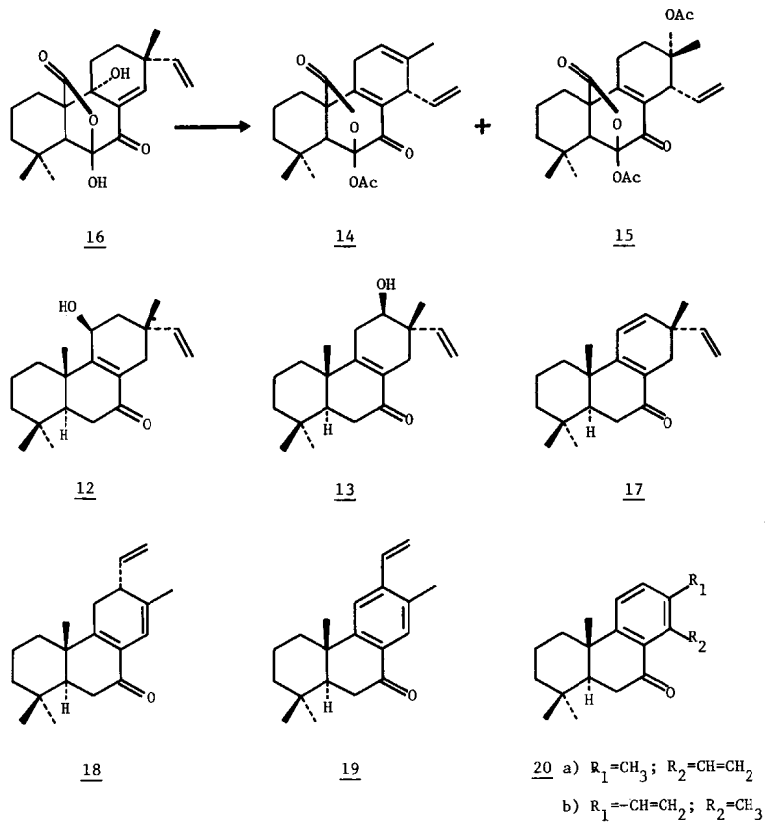
The absolute configurations of the other *Vellozia* cleistanthane diterpenes were deduced by correlation with **9** in the following way. LAH reduction of the methyl ester of **8** furnished a primary alcohol spectroscopically identical to veadeirol (**7**).[7] Comparison of the optical rotations of natural **7** with the reduction product of the acid showed both to be dextrorotary thus proving that veadeiroic acid (**8**) and veadeirol (**7**) had the same absolute configuration. Hydrogenolysis of **7** furnished hydrocarbon **11** ($[\alpha]_D = +41.0°$) which, on oxidation with t-butyl chromate, afforded cleistantha-8, 11, 13-trien-7-one, identical spectroscopically and by optical rotation with natural **9**. Consequently, all the cleistanthanes reported here (**7–9**) and isolated from *Velloziaceae* have the (5S, 10S) absolute configuration and belong thus to the antipodal series of cleistanthanes as those obtained from the *Euphorbiaceae* and *Compositae*.

As mentioned above, *Velloziaceae* are also rich sources of pimarane diterpenes.[8] We reported previously the structure elucidation (relative configuration only) of 11β-hydroxy-isopimara-8, 15-dien-7-one (**12**) and 12β-hydroxy-isopimara-8, 15-dien-7-one (**13**) both isolated from *Vellozia compacta*.[12] Their absolute configurations are now reported. The CD curves of **12** ($[\theta]_{326} = +7.830$) and **13** ($[\theta]_{326} = +7.220$), in dioxane showed a positive Cotton effect consistent with the (5S, 10S) absolute configuration[11] of the A/B ring junction carbon atoms. Hence it appears that in *Velloziaceae*, pimaranes and cleistanthanes (at least those reported here) possess the same (5S, 10S) absolute configuration. Similarly, in *Euphorbiaceae*, pimaranes and cleistanthanes have the same (5R, 10R) absolute configuration.[6] These facts are in good agreement with the proposal that pimaranes are the biogenetic precursors of cleistanthanes.[6] Experimental support to this hypothesis came from the formation of cleistanthanes **14** and **15** by treatment of pimarane **16** with acetic anydride and catalytic amount of TsOH.[13]

This prompted us to try to correlate pimarane **13** to the cleistanthane series via a biomimetic rearrangement. Treatment of **13** with TsOH in benzene yielded only the dehydration product **17**.[14] When $POCl_3$ was used as



Scheme 1.



Scheme 2.

dehydrating agent, a mixture of two compound was formed. One is again the dehydrated derivative **17**; the other, **18**, resulted from loss of the oxygen function at C-12 and subsequent migration of the vinyl group from C-13 to C-12. **18** was rather unstable and was readily oxidized to **19** which was easily identified by its spectral data. Although it was not the desired rearrangement, the obtention of **18** (and **19**) constitutes the first example of migration, in the isopimarane series, of a vinyl group from C-13 to C-12. As far as we know, there is no report in the literature of a natural diterpene having the skeleton of **19**. Finally, refluxing **13** in benzene in the presence of NBS, two less polar products were obtained; one was identical to **17**, the other is an aromatic compound (UV: 262 (log ϵ = 3.49) and 229 nm (log ϵ = 3.60)) bearing a ketone at the benzylic position (IR: 1685 cm^{-1}). Its ^1H NMR spectrum showed the presence of three methyl groups on quaternary carbons, and of a methyl and a vinyl group on a tetrasubstituted aromatic ring. The AB system at 7.21 (1H d J = 8 Hz) and 7.38 ppm (1H d J = 8 Hz) proved the *ortho* relationship of the hydrogens on the aromatic nucleus a conclusion also supported by the IR absorption at 825 cm^{-1}. These data were compatible either with structure **20a** (resulting from the migration of the vinyl group) or with structure **20b** (resulting from the migration of the methyl group). Structure **20a** was shown to be the correct one by catalytic hydrogenation to **9** identical with an authentic sample. Since natural and synthetic **9** were both dextrorotatory, this reaction not only consistituted a biomimetic transformation of an isopimarane into a cleistanthane diterpene, but also confirmed both series of *Vellozia* diterpene to have identical absolute configuration.

EXPERIMENTAL

M.ps were determined on a Köfler hotstage and are uncorrected. Optical rotations were measured with a Perkin–Elmer 241 polarimeter at ambient temperature (~25°) in $CHCl_3$ solutions. ORD and CD spectra were recorded at ambient temperature on a Fica Spectropol-1 instrument. IR spectra were recorded in KBr pellets with a Perkin–Elmer 137 apparatus. Low resolution mass spectra (MS) were determined on a VG Micromass MM12F instrument; intensities of the fragments are expressed as percentages of the base peak (100%). ^1H NMR (100 MHz) and ^{13}C NMR (25, 2 MHz) spectra were recorded with a Varian XL-100 apparatus; unless otherwise mentioned, spectra were recorded in $CDCl_3$ solutions using tetramethylsilane as internal reference; shifts are expressed in the δ scale; the following abreviations were used: b = broad, d = doublet, m = multiplet, q = quadruplet, s = singlet and t = triplet. UV spectra were recorded in MeOH solutions with a Beckman DB-GT grating spectrophotometer coupled to a Beckman 10-in. recorder. Analytical and preparative thin layer chromatographies (TLC) were performed on E. Merck Kieselgel 60 PF plates, the analytical chromatograms being revealed by UV light (λ_{254} nm) and by spraying a 0.2% solution of ceric sulfate in 2N aqueous H_2SO_4 followed by heating on a hot plate. Column chromatography was performed on E. Merck Kieselgel 60 (70–230 mesh).

Isolation of cleistantha-8, 11–13-*trien*-7-*one* (**9**)

Cleistantha-8, 11, 13, trien-7-one (**9**) was isolated from the hexane crude extract (43 g) of roots, stems and leaf sheats of *Vellozia leptopetala* by silica gel column chromatography eluted with pure hexane (yield: 3% from crude extract) **9**: m.p. 89–90°; $[\alpha]^{25}$ +35.5(589), +39.8(578), +55.5(546) and +374.7°(436 nm), c = 1.00, $CHCl_3$; UV: 222 (log ϵ = 3.78), 257 (log ϵ = 3.84) and 305 nm (log ϵ = 3.29); IR: 2872, 1675, 1450, 1347, 1132, 1052, 981, 952, 938 and 826 cm^{-1}; MS: $M^{+\cdot}$ 284 (100, $C_{20}H_{28}O$), 269 (23), 251 (47), 201 (40), 199 (48), 187 (63), 181 (30) and 173 (30); ^1H NMR: 0.90 (3H, s C-19 Me), 1.00 (3H, s C-18 Me), 1.14 (3H, s C-20 Me), 1.21 (3H, t J = 7 Hz C-16 Me), 1.77 (1H, t J = 8 Hz 5αH), 2.30 (3H, s C-17 Me), 2.66 (2H, d J = 8 Hz C-6 H_2), 2.91 (2H, q J = 7 Hz C-15 H_2), 7.17 and 7.22 ppm (each 1 H J = 7 Hz C-11 and C-12 H); ^{13}C NMR: 201.7 (s), 154.4 (s), 143.8 (s), 134.9 (s), 134.3 (d), 130.9 (s), 120.2 (d), 47.8 (d), 41.7 (t), 38.4 (2t), 38.0 (s), 33.2 (s), 32.2 (q), 23.6 (t), 23.1 (q), 21.3 (q), 19.1 (q), 18.9 (t) and 14.5 ppm (q); ORD (c = 0.1 mg/ml cyclohexane): [Φ] = +4.360(367), 0(345) and −13.080(313 nm); CD(c = 7.7 × 10^{-4} M, cyclohexane $[\theta]_{336}$ = +4.005.

LAH reduction of veadeiroic acid **8**)

Veadeiroic acid **8** (20 mg) was methylated with diazomethane in ether at room temperature. After evaporation of the ether, the crude ester obtained (20.9 mg) was dissovled in dry THF (2 ml) and treated with LAH (10 mg) at room temperature for 4 hr. The usual work up of the reaction medium furnished pure veadeirol **7** (18 mg), identical by spectroscopy and $|\alpha|$ with an authentic sample.[7]

Hydrogenolysis of veadeirol (**7**)

Veadeirol **7** (20.3 mg) in AcOH (20 ml) was treated at room temperature with hydrogen (62 psi) in the presence of 10% Pd/C. After 1 hr 30, the catalyst was filtered off and washed with AcOEt. Evaporation of the filtrate under reduced pressure yielded 19 mg of cleistantha-8, 11, 13-triene (**11**) as a colorless oil. **11**: m.p. 58–59°; [α] = +41.0(589), +42.6(578), +48.3(546), +80.1(436) and +121.6°(365 nm) (c = 1.00, $CHCl_3$); UV 226 (log ϵ = 3.30) and 2.70 (log ϵ = 2.16); IR: 2875, 1455, 1350 and 820 cm^{-1}; MS: $M^{+\cdot}$ 270.2371 (33, $C_{20}H_{30}$; Calc. 270.2347), 256(15), 255(72), 199(14), 185(57), 173(56), 159(100), 69(35), 41(23); ^1H-NMR: 0.94 (3H, s), 0.97 (3H, s), 1.19 (3H, t J = 7 Hz), 1.22 (3H, s), 2.30 (3H, s), 2.64 (2H, q J = 7 Hz), 2.82 to 3.00 (1H, m), 6.97 (1H, d J = 8 Hz) and 7.09 ppm (1H, d J = 8 Hz).

Oxidation of cleistantha-8, 11, 13-*triene* (**11**)

To a solution of cleistantha-8, 11, 13-triene (**11**) (19 mg) in CCl_4 (2 ml) were added under stirring AcOH (0.7 ml), Ac_2O (0.4 ml) and t-butyl chromate (0.4 ml). The reaction medium was refluxed during 4 hr, after what the solvents were evaporated under reduced pressure. The product was extracted successively two times with a saturated aqueous solution of oxalic acid, chloroform and water. Evaporation of the chloroform layer furnished 16.3 mg of a yellowish oil. Crystallization from hexane yielded a colorless solid identical to **9** by ^1H NMR and optical rotation.

ORD and CD spectra of pimaranes **12** and **13**

12: CD (1.14 × 10^{-3} M in dioxane); [θ] = +7.830 at 326 nm; **13**: ORD (0.113 mg/ml in dioxane) [Φ] = +3.204(366) 0(337) and −4.305°(307 nm) CD (3.74 × 10^{-4} M in dioxane): [θ] = +7.220 at 326 nm.

Treatment of 12β-*hydroxyisopimara*-8, 15-*dien*-7-*one* (**13**) *with* $POCl_3$

To a soln of 12β-hydroxy-isopimara-8, 15-dien-7-one (**13**; 110 mg) in pyridine (2 ml), cooled to 0°, was added $POCl_3$ (0.4 ml). After 5 min, the reaction medium was added water (10 ml), and extracted with CH_2Cl_2 (15 ml). The organic layer was washed with water (2 × 10 ml), dried over Na_2SO_4, filtered and evaporated to dryness. The crude reacted mixture (90 mg) was purified by silica gel preparative TLC (hexane–AcOEt = 9 : 1), yielding 21 mg of isopimara-8, 11, 15-trien-7-one (**17**) identical with an authentic sample[14] and 18 mg of 15(13→12β H)-*abeo*-isopimara-8, 13, 15-trien-7-one (**18**). This compound readily oxidized after 15 days in the air to 15(13→12)-*abeo*-isopimara-8, 11, 13, 15-tetraen-7-one (**19**). **18**: IR: 1650, 1610, 1585, 1370, 990, 910 and 875 cm^{-1}; MS: $M^{+\cdot}$ 284 (16, $C_{20}H_{28}O$), 161 (100); ^1H NMR (CCl_4): 0.95 (6H, s), 1.14 (3H, s), 1.78 (3H, bs), 4.95 (1H, dd, J = 10 and 1.5 Hz), 5.00 (1H, dd J = 18 and 1.5 Hz), 5.59 (1H, dd J = 18 and 10 Hz) and 6.25 ppm (1H, bs). The latter signal was sharpened on irradiation at 1.78 ppm. **19**: UV: 283 (log ϵ = 4.09) and 218 nm (log ϵ = 4.00); IR: 1675, 1620, 1600, 1400, 1375, 1275, 1200, 990, 935, 910 and 900 cm^{-1}; MS: $M^{+\cdot}$ 282 (55, $C_{20}H_{26}O$), 267 (100), 197 (72), 185 (60) and 171 (28); ^1H NMR (CCl_4): 0.99 (3H,

s), 1.03 (3H, s), 1.27 (3H, s), 2.36 (3H, s), 5.33 (1H, dd J = 11 and 1.5 Hz), 5.61 (1H, dd J = 18 and 1.5 Hz), 6.89 (1H, dd J = 18 and 11 Hz), 7.33 (1H, s) and 7.66 ppm (1H, s). The latter signal was intensified on irradiation at 2.36 ppm.

Treatment of 12β-hydroxyisopimara-8, 15-dien-7-one (**13**) with NBS

A soln of *12β-hydroxyisopimara-8, 15-dien-7-one* (**13**; 91 mg), in benzene (4 ml), was refluxed during 12 hr 30 with NBS (57 mg). The reaction medium was diluted with CH_2Cl_2 (20 ml), washed with a 8% aqueous solution of $NaHCO_3$ (15 ml) and then with water (3 × 15 ml). The organic layer was dried over Na_2SO_4, filtered off and evaporated under reduced pressure. The crude extract (90 mg) was chromatographed on a silica gel TLC plate (eluent; hexane–AcOEt, 9:1) yielding 17 mg of isopimara-8, 11, 15-trien-7-one (**17**) identical with an authentic sample[14] and 22 mg of cleistantha-8, 11, 13, 15-tetraen-7-one (**20a**), UV: 262 (log ε = 3.49) and 229 (log ε = 3.60); IR: 1680, 1480, 1450, 1385, 1275, 1205, 1110, 935, 910 and 825 cm^{-1}; MS: $M^{+\cdot}$ 282 (91, $C_{20}H_{26}O$), 281 (100), 197 (23), 185 (14) and 171 (11); ^1H NMR: 0.94 (3H, s), 1.02 (3H, s), 1.19 (3H, s), 2.35 (3H, s), 5.07 (1H, dd, J = 18 and 1.5 Hz), 5.44 (1H, dd, J = 11 and 1.5 Hz), 7.08 (1H, dd, J = 18 and 11 Hz), 7.21 (1H, d, J = 8 Hz) and 7.38 ppm (1H, d, J = 8 Hz).

Hydrogenation of cleistantha-8, 11, 13, 15-tetraen-7-one (**20a**)

A soln of cleistantha-8, 11, 13, 15-tetraen-7-one (**20a**) (18 mg) in AcOEt (2 ml) was hydrogenated over 10% Pd/C (1 mg) at a pressure of 30 psi. After 4 hr, the catalyst was filtered off and the filtrate evaporated under reduced pressure to yield a crystalline compound (18 mg) identical by mp, [α], UV, IR, MS and ^1H NMR with natural cleisthantha-8, 11, 13, 15-trien-7-one **9**) (see above).

Acknowledgements—We are grateful to Prof. Nanusa L. de Menezes (Department of Botany, USP, Brazil) for the identification of the *Vellozia* species. Specific rotations were measured on the spectrophotometer of the laboratories Silva Araujo Roussel S. A. This work was supported by FINEP, CNPq and CAPES–UFRJ funds.

REFERENCES

[1]T. K. Devon and A. I. Scott, *Handbook of Naturally Occurring Compounds*, Vol. II, pp 213–217. Academic Press, New York (1972), and refs cited therein.
[2]E. J. McGarry, K. H. Pegel, L. Phillips and E. S. Waight, *J. C. S. Chem. Commun.* 1074 (1969).
[3]A. A. Craveiro and E. R. Silveira, *Ciência e Cultura (Supl.)* **31**, 395 (1979). *Phytochemistry* **21**, 2571 (1982).
[4]S. P. Gunasekara, G. A. Cordelly and N. R. Farnsworth, *J. Natl Prod. (Lloydia)* **42**, 658 (1979).
[5]F. Bohlmann, M. Bapuji, J. Jakupovic, R. M. King and M. Robinson, *Phytochemistry* **21**, 181 (1982).
[6]E. J. MacGarry, K. M. Pegel, L. Phillips and E. S. Waight, *J. Chem. Soc. (C)* 904 (1971).
[7]R. Pinchin, L. M. U. Mayer and A. C. Pinto, *Phytochemistry* **17**, 1671 (1978).
[8]A. C. Pinto, W. S. Garcez, R. S. Silva, L. M. M. Valente, E. M. Peixoto, P. P. S. Queiroz and A. L. Pereira, *J. Chem. Res. (M)* 1701 (1982); *Ibid. (S)* 154 (1982) and refs cited therein.
[9]P. P. S. Queiroz, W. S. Garcez, E. M. Peixoto and A. C. Pinto, in *VI Symp. on Brazilian Med. Plants*, Fortaleza, *Ciência e Cultura (Supl.)* 109 (1980).
[10]R. C. Cambie, L. N. Mander, A. K. Bose and M. S. Manhas, *Tetrahedron* **20**, 409 (1974).
[11]G. Snatzke, In *Optical Rotatory Dispersion and Circular Dichroism in Organic Chemistry*, Chap. 13. Heyden, London (1967).
[12]A. C. Pinto and C. Borges, *Phytochemistry* in press.
[13]G. A. Ellestand, M. R. Kunstmann and G. O. Morton, *J. Chem. Soc. (C)* 312 (1973).
[14]A. C. Pinto, L. M. M. Valente, R. S. Silva, W. S. Garcez and P. P. S. Queiroz, *An. Acad. brasil. Ciênc.* **53**, 73 (1981).