Phytochemistry, Vol. 21, No. 10, pp. 2563–2566, 1982.
Printed in Great Britain.

0031–9422/82/102563–04$03.00/0

SALVIACOCCIN, A NEO-CLERODANE DITERPENOID FROM *SALVIA COCCINEA*

GIUSEPPE SAVONA, MAURIZIO BRUNO, MARIAPIA PATERNOSTRO, JOSÉ L. MARCO* and BENJAMÍN RODRÍGUEZ*

Istituto di Chimica Organica dell'Università, Archirafi 20, 90123-Palermo, Italy; *Instituto de Quimica Orgánica, CSIC, Juan de la Cierva 3, Madrid-6, Spain

(*Received* 9 *February* 1982)

Key Word Index—*Salvia coccinea*; Labiatae; neo-clerodane derivative; salviacoccin; 15, 16-epoxy-10-hydroxy-neo-cleroda-2,7,13(16), 14-tetraene-17, 12*R*: 18,19-diolide.

Abstract—A new neo-clerodane diterpenoid, salviacoccin, was isolated from the aerial part of *Salvia coccinea*. Its structure, 15,16-epoxy-10-hydroxy-neo-cleroda-2,7,13(16), 14-tetraene-17,12*R*:18,19-diolide, was established by chemical and spectroscopic means and by comparison with closely related compounds.

INTRODUCTION

In our search for new natural substances in the *Salvia* genus (Labiatae family)[1–4], we have examined the aerial part of *S. coccinea*, a species originating from Central and South America, the triterpenic constituents of which have been previously studied[5]. From this plant we have now isolated a novel diterpenoid, salviacoccin, the structure of which has been shown to be 15,16-epoxy-10-hydroxy-neo-cleroda-2, 7, 13(16), 14-tetraene-17, 12*R*:18, 19-diolide (**1**) by chemical and spectroscopic means, and in accordance with the nomenclature proposed by Rogers *et al.*[6].

RESULTS AND DISCUSSION

Combustion analysis and mass spectrometry indicated the molecular formula $C_{20}H_{20}O_6$ for salviacoccin (**1**). Its IR spectrum was consistent with the presence of a furan ring (3160, 3120, 1505, 880 cm^{-1}), olefinic double bonds (3040, 3030, 3010, 1655 cm^{-1}), a γ-lactone group (1770 cm^{-1}), another lactone group, probably an α,β-unsaturated δ-lactone (1708 and 1655 cm^{-1})[7, 8] and a hydroxyl group (strong and sharp band at 3460 cm^{-1}). The presence of a furan ring and an α,β-unsaturated lactone group were also revealed by the UV spectrum of compound **1**, which showed typical absorptions for these two chromophores [λ_{max}^{EtOH} nm (ϵ): 215 (6700), 220 sh (5700), 234 sh (1400), 245 sh (800)][7–9].

However, it was the ^{1}H NMR spectrum of salviacoccin (**1**) that provided the most information. It showed signals of a tertiary methyl group at δ 1.00 (a singlet), of a β-substituted furan ring [A_2X system, two α-furan protons having their resonance at 7.57 (*m*, $W_{1/2}$ = 5 Hz), and one β-furan proton at 6.58 (*m*, $W_{1/2}$ = 4.5 Hz)], of an olefinic proton at 6.70 (*dd*, J_1 = 4.2 Hz, J_2 = 3 Hz), which can be assigned to an olefinic β-proton of an α,β-unsaturated lactone group[7–10], and of two more olefinic protons at 5.92 (*m*, $W_{1/2}$ = 14 Hz) and 5.75 (*dd*, J_1 = 11 Hz, J_2 = 3 Hz). The following signals due to three protons on carbon atoms bearing oxygen atoms could also be seen: 5.33 (1H, *dd*, J_1 = 12 Hz, J_2 = 3 Hz), 4.13 and 4.03 (an AB system, J_{AB} = 9 Hz, in which the signal at 4.13 showed an additional long-range coupling, J = 1.5 Hz). In addition, the ^{1}H NMR spectrum of compound **1** showed three one-proton double doublets at 3.21 (J_1 = 4.5 Hz, J_2 = 3 Hz), 2.85 (J_1 = 14 Hz, J_2 = 3 Hz) and 1.75 (J_1 = 14 Hz, J_2 = 12 Hz), as well as a singlet at 6.48, which disappeared on D_2O exchange and must be assigned to a tertiary hydroxyl group[7].

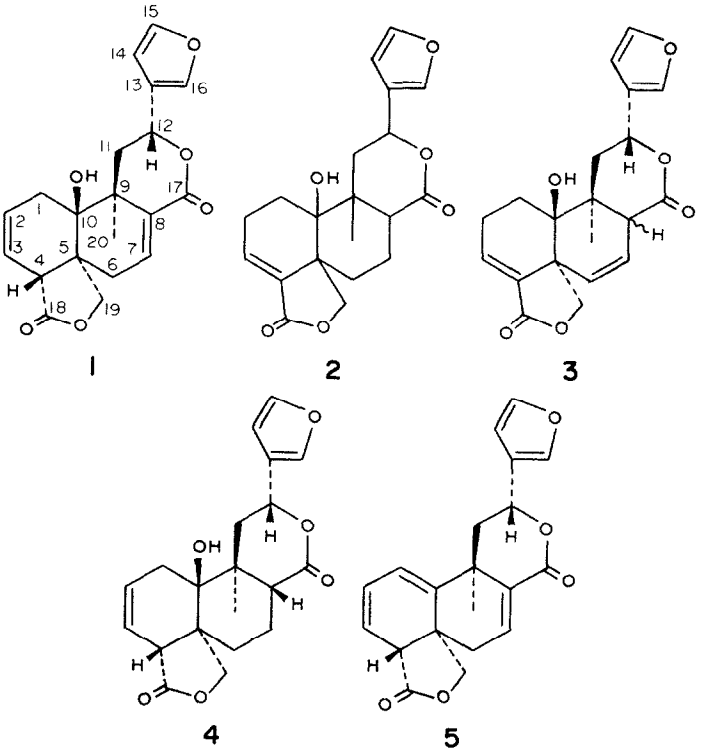
In order to establish the relative arrangement of the protons, a series of proton decoupling experiments was carried out. As a result of irradiation at δ 5.33, the double doublet signals at 2.85 and 1.75 collapsed into an AB system (J = 14 Hz). Irradiation at 5.82 (centre of the signals of the two olefinic protons) converted the signal at 3.21 into a singlet. Furthermore, by irradiating at 6.70 (the β-proton of an α,β-unsaturated lactone group) no variation was observed in any of the well-defined signals, but a complex signal appearing at 2.40 (4H) was partially modified.

On the basis of these results, the following assignment for the protons could be made, which are in agreement with structure **1** for salviacoccin. The signal at δ 6.70 was assigned to the C-7 proton ($J_{7,6\beta}$ = 4.2 Hz, $J_{7,6\alpha}$ = 3 Hz, see Dreiding molecular model of compound **1**) and the signals of the C-6 protons appeared at *ca* 2.40[7, 8]. The signal at 5.33 was assigned to the C-12β axial proton ($J_{12\beta,11\alpha}$ = 12 Hz, $J_{12\beta,11\beta}$ = 3 Hz) and the double doublets at 2.85 ($J_{11\beta,11\alpha}$ = 14 Hz, $J_{11\beta,12\beta}$ = 3 Hz) and 1.75 ($J_{11\alpha,11\beta}$ = 14 Hz, $J_{11\alpha,12\beta}$ = 12 Hz) were attributed to the C-11 equatorial and C-11 axial protons, respectively[1, 2, 7–9, 11–13]. The AB system at 4.13 and 4.03 (J = 9 Hz) was assigned to the C-19 methylene grouping, one of the protons of this methylene group is in turn *W* coupled with the C-6β proton ($^4J_{19A,6\beta}$ = 1.5 Hz). This behaviour has been previously found in several clerodan-18,19-olides possessing a C-6 methylene

group[2, 9], but not in compounds with a C-6β hydroxyl group[10] or a C-6 sp^2 carbon atom[14]. Thus, it is evident that salviacoccin possesses a methylene group in the C-6 position. Finally, the double doublet at 3.21 was assigned to the C-4β proton, which is coupled with the C-3 olefinic proton at 5.75 ($J_{4,3}$ = 3 Hz) and also with the C-2 olefinic proton at 5.92 ($^4J_{4,2}$ = 4.5 Hz). The same behaviour has been previously observed for closely related structures such as salviarin[1] and splendidin[2].

In the proposed structure for salviacoccin (**1**), it is evident that its tertiary hydroxyl group must be placed on the C-10 position. Thus, this new diterpenoid may be closely related to a compound (**2**) previously isolated from *Salvia rubescens*[15], a species botanically close to *S. coccinea*.

On the other hand, treatment of salviacoccin (**1**) with thionyl chloride yielded the dehydro derivative **5** ($C_{20}H_{18}O_5$), which showed UV absorptions due to a diene system [λ_{max}^{EtOH} nm (ε): 264 (4200), 272 sh (4000) and 283 sh (2600)] identical with those reported for the 10(1),2-dienic derivative of compound **2**[15]. The ^{1}H NMR spectrum of compound **5** was also in agreement with this structure, because the following signals were encountered: δ 6.00 (1H, *d*, $J_{1,2}$ = 6 Hz, H-1), 6.17 (1H, *ddd*, $J_{2,1}$ = 6 Hz, $J_{2,3}$ = 9 Hz, $J_{2,4}$ = 3 Hz, H-2), 5.59 (1H, *dd*, $J_{3,2}$ = 9 Hz, $J_{3,4}$ = 3 Hz, H-3), 3.18 (1H, *dd*, $J_{4,3} = J_{4,2}$ = 3 Hz, H-4) and 6.78 (1H, *dd*, $J_{7,6\alpha} = J_{7,6\beta}$ = 4.2 Hz, H-7) (see also the Experimental). This result firmly established that the tertiary hydroxyl group of salviacoccin is in the C-10 position. The β-configuration assigned to this tertiary hydroxyl



All the above data may be also, but less probably, accomodated on a structure such as **3**, in which the signal at δ 3.21 could be attributed to the C-8 proton. However, structure **3** was discarded for salviacoccin and the structure and absolute configuration **1** confirmed on the basis of the following considerations.

Reduction of salviacoccin with sodium borohydride yielded a $C_{20}H_{22}O_6$ compound (**4**), in which the conjugated olefinic double bond was the one hydrogenated[10, 15]. The IR spectrum of compound **4** showed lactonic absorptions at 1770 (γ-lactone) and 1725 cm^{-1} (δ-lactone), instead of the bands at 1770 and 1708 cm^{-1} of salviacoccin (**1**). Thus, the α,β-unsaturated lactone of the new diterpenoid is the δ-lactone. The C-8 hydrogen atom in compound **4** is axial (β), because it appeared in the ^{1}H NMR spectrum as a double doublet at δ 3.61 ($J_{8\beta,7\beta}$ = 6 Hz, $J_{8\beta,7\alpha}$ = 9 Hz[9].

group in the new diterpenoid (**1**) needs, in our opinion, a rigorous justification, since it is an unusual feature in the more common 5α–10βH–9α–8β backbone relationship of the neo-clerodane diterpenoids. This was confirmed as follows. (1) When the signal of the C-20 methyl group in the ^{1}H NMR spectrum of salviacoccin (**1**) was irradiated, it gave a clear Overhauser effect (*ca* 20%) on the C-19 methylene signals, and vice versa. Thus, both substituents are axially oriented. A structure with a C-10α-hydroxyl group does not justify this observation (see the molecular model of compound **1**). (2) The 8β-axial proton of compound **4** appeared at δ 3.61 (see above), whereas the same proton appeared at δ 2.68 in bacchotricuneatin A, a neo-clerodane diterpenoid closely related to compound **4** but with a 10β-H substituent[9]. This chemical shift difference is due to the 1,3-diaxial interactions caused by the 10β-hydroxyl group of the dihydro derivative **4**. (3) Com-

Table 1. ^{13}C NMR chemical shifts (in δ values from TMS) of compound **1***

C-1	29.2 *t*†	C-11	40.6 *t*
C-2	126.8 *d*	C-12	72.9 *d*
C-3	121.1 *d*	C-13	124.7 *s*
C-4	51.2 *d*	C-14	109.2 *d*
C-5	45.8 *s*	C-15	143.8 *d*
C-6	34.3 *t*	C-16	140.3 *d*
C-7	132.6 *d*	C-17	169.2 *s*
C-8	135.9 *s*	C-18	175.4 *s*
C-9	41.2 *s*	C-19	73.7 *t*
C-10	72.5 *s*	C-20	28.1 *q*

*In pyridine-d_5 solution.
†SFORD multiplicity.

parison between the ^{13}C NMR spectra of salviacoccin (**1**) (Table 1) and salviarin[1] further confirmed this point. Effectively, only a 10β-hydroxyl configuration in compound **1** explains the downfield shift on its C-19 ($\Delta\delta + 3.7$) and C-20 ($\Delta\delta + 4.4$) carbon atoms with respect to salviarin[1], whereas for a 10α-hydroxyl group shielding effects are expected for these carbon atoms[16].

Finally, the neo-clerodane[6] absolute configuration of salviacoccin (**1**) was inferred from the CD curve of compound **5**, which showed a positive Cotton effect ($\Delta\epsilon_{292} + 1.08$) associated to the *cisoid* diene with a right ellipticity[17] (see the molecular model of salviacoccin).

EXPERIMENTAL

Mps were determined on a Kofler apparatus and are uncorr. Elemental analyses were carried out in Madrid with the help of an automatic analyser. Assignments of ^{13}C chemical shifts were made with the aid of off-resonance and noise-decoupled ^{13}C NMR spectra. Plant materials were collected in June 1980 in the Botanic Garden of Palermo, Italy, and voucher specimens were deposited in the Herbarium of this Centre.

Extraction and isolation of the diterpenoid. Dried and finely powdered aerial parts (450 g) of *Salvia coccinea* Juss. were extracted with Me_2CO (5 l.) at room temp. for 1 week. After filtration the solvent was evaporated yielding a gum (23 g) which was subjected to dry-CC over Si gel (400 g, Merck No. 7734, deactivated with 15% H_2O). Elution with petrol and petrol–EtOAc (4:1) gave plant waxes and phytosterols which were rejected. Elution with EtOAc–petrol (2:1) yielded salviacoccin (**1**, 250 mg) which was crystallized from MeOH: mp 242–244°;$[\alpha]_D^{20} - 139.8°$ (C_5H_5N; *c* 0.49): IR ν_{max}^{KBr} cm^{-1}: 3460 (OH); 3160, 3120, 1505, 880 (furan ring); 3040, 3030, 3010 (olefinic hydrogens); 1770 (γ-lactone); 1708, 1655 (α,β-unsatd δ-lactone); 2980, 2950, 2920, 1430, 1380, 1365, 1280, 1260, 1230, 1180, 1165, 1140, 1050, 1035, 1015, 995, 850, 810, 800, 755, 690, 675. UV λ_{max}^{EtOH} nm (log ϵ): 215 (3.83), 220 sh (3.76), 234 sh (3.15), 245 sh (2.90) (furan ring and α,β-unsatd δ-lactone). ^1H NMR (90 MHz, C_5H_5N-d_5 and C_5H_5N-d_5 plus D_2O): see the Results and Discussion; Overhauser effect, irradiation of the signal at δ 1.00 causes an NOE of 19.5% on the δ 4.13 and 4.03 signals and vice versa. ^{13}C NMR (25.2 MHz, C_5H_5N-d_5): see Table 1. EIMS (direct inlet) 75 eV, *m/z* (rel. int.): 356 $[M]^+$ (30), 338 (25), 310 (44), 245 (8), 229 (100), 141 (24), 129 (23), 128 (22), 114 (31), 107 (23), 95 (67), 94 (43), 91 (45), 81 (60), 79 (55), 77 (48), 65 (38), 53 (46), 51 (39), 43 (33), 41 (55). (Found: C, 67.12; H, 5.61. $C_{20}H_{20}O_6$ requires: C, 67.40; H, 5.66%.)

7,8β-Dihydrosalviacoccin (**4**). To a soln of salviacoccin (**1**, 22 mg) in dioxane–MeOH (1:1, 4 ml) excess of $NaBH_4$ was added and the soln was stirred at room temp. for 30 min. The excess of reagent was then destroyed by addition of Me_2CO. Work-up in the usual manner yielded compound **4** (18 mg, after crystallization from Me_2CO–*n*-hexane): mp 268–270°; $[\alpha]_D^{27} - 127.5°$ (C_5H_5N; *c* 0.345); IR ν_{max}^{KBr} cm^{-1}: 3500 (OH); 3150, 3140, 1505, 875 (furan ring); 3035, 3010 (olefinic double bond); 1770 (γ-lactone); 1725 (δ-lactone); 2980, 2940, 2860, 1480, 1450, 1385, 1370, 1300, 1245, 1205, 1185, 1160, 1055, 1020, 1010, 985, 930, 850, 820, 805, 770, 730, 700, 670. UV λ_{max}^{EtOH} nm (log ϵ):215 (3.81) (furan ring. ^1H NMR (90 MHz, C_5H_5N-d_5): δ 7.65 (2H, *m*, $W_{1/2}$ = 4.5 Hz, H-15 and H-16), 6.65 (1H, *m*, $W_{1/2}$ = 4 Hz, H-14), 6.38 (1H, *s*, disappeared on D_2O exchange, –OH), 5.96 (1H, *m*, $W_{1/2}$ = 14 Hz, H-2), 5.80 (1H, *dd*, $J_{3,2}$ = 10.5 Hz, $J_{3,4}$ = 3 Hz, H-3), 5.57 (1H, *dd*, $J_{12\beta,11\beta}$ = 4.5 Hz, $J_{12\beta,11\alpha}$ = 12 Hz, axial H-12), 4.40 (1H, *d*, $J_{19B,19A}$ = 9 Hz, H_B-19, endo hydrogen respect ring B), 4.17 (1H, *dd*, $J_{19A,19B}$ = 9 Hz, $J_{19A,6\beta}$ = 1 Hz, H_A-19, exo hydrogen respect ring B), 3.61 (1H, *dd*, $J_{8\beta,7\alpha}$ = 9 Hz, $J_{8\beta,7\beta}$ = 6 Hz, axial H-8), 3.20 (1H, *dd*, $J_{4,3} = J_{4,2}$ = 3 Hz, H-4), 2.87 (1H, *dd*, $J_{11\beta,11\alpha}$ = 14 Hz, $J_{11\beta,12\beta}$ = 4.5 Hz, equatorial Hβ-11), 1.70 (1H, *dd*, $J_{11\alpha,11\beta}$ = 14 Hz, $J_{11\alpha,12\beta}$ = 12 Hz, axial Hα-11) and 0.93 (3H, *s*, 3H-20); all these assignments were confirmed by double resonance expts. EIMS (direct inlet) 10 eV, *m/z* (rel. int.): 358 $[M]^+$ (100), 340 (23), 312 (8), 296 (6), 262 (6), 260 (4), 244 (19), 230 (37), 219 (12), 202 (8), 164 (3), 158 (4), 121 (5), 94 (34). (Found: C, 66.92; H, 6.31. $C_{20}H_{22}O_6$ requires: C, 67.02; H, 6.19%.)

1(10)-Dehydrosalviacoccin (**5**). To a soln of salviacoccin (**1**, 30 mg) in dry C_5H_5N (3 ml) cooled at 0°, five drops of freshly distilled $SOCl_2$ were added. The soln was kept at 0° for 30 min. and then at room temp. (5 hr). Work-up in the usual manner yielded 19 mg of compound **5** (after two crystallizations from Me_2CO–*n*-hexane: mp 182–183.5°; $[\alpha]_D^{20} - 186.4°$ ($CHCl_3$; *c* 0.368); IR ν_{max}^{KBr} cm^{-1}: 3150, 3130, 1505, 880 (furan ring); 3065, 3050, 1580 (dienic system); 1775 (γ-lactone); 1715, 1670 (α,β-unsatd δ-lactone); 2990, 2970, 2920, 2885, 1465, 1430, 1370, 1280, 1245, 1170, 1060, 1040, 1010, 905, 855, 795, 770, 750, 710, 655. UV λ_{max}^{EtOH} nm (log ϵ): 212.5 (3.88) (furan ring); 220 sh (3.77) (α,β-unsatd δ-lactone); 264 (3.62), 272 sh (3.60), 283 sh (3.41) (*cisoid* diene). CD: $\Delta\epsilon_{285} = 0$, $\Delta\epsilon_{292} = +1.08$, $\Delta\epsilon_{323} = 0$ (EtOH; *c* 0.59). ^1H NMR (90 MHz, $CDCl_3$): δ 7.48 (1H, *m*, $W_{1/2}$ = 4 Hz, H-16), 7.43 (1H, *dd*, $J_{15,16} = J_{15,14}$ = 1.8 Hz, H-15), 6.78 (1H, *dd*, $J_{7,6\alpha}$ = $J_{7,6\beta}$ = 4.2 Hz, H-7), 6.46 (1H, *m*, $W_{1/2}$ = 4 Hz, H-14), 6.17 (1H, *ddd*, $J_{2,1}$ = 6 Hz, $J_{2,3}$ = 9 Hz, $J_{2,4}$ = 3 Hz, H-2), 6.00 (1H, *d*, $J_{1,2}$ = 6 Hz, H-1), 5.59 (1H, *dd*, $J_{3,2}$ = 9 Hz, $J_{3,4}$ = 3 Hz, H-3), 5.12 (1H, *dd*, $J_{12\beta,11\alpha}$ = 10.2 Hz, $J_{12\beta,11\beta}$ = 4.8 Hz, axial H-12), 4.33 (1H, *d*, $J_{19B,19A}$ = 8.4 Hz, H_B-19, endo hydrogen respect ring B), 4.11 (1H, *dd*, $J_{19A,19B}$ = 8.4 Hz, $J_{19A,6\beta}$ = 1.2 Hz, H_A-19, exo hydrogen respect ring B), 3.18 (1H, *dd*, $J_{4,3} = J_{4,2}$ = 3 Hz, H-4), 2.73 (1H, *dd*, $J_{6\alpha,6\beta}$ = 19.2 Hz, $J_{6\alpha,7}$ = 4.2 Hz, Hα-6), 2.35 (1H, *ddd*, $J_{6\beta,6\alpha}$ = 19.2 Hz, $J_{6\beta,7}$ = 4.2 Hz, $J_{6\beta,19A}$ = 1.2 Hz, Hβ-6), 2.63 (1H, *dd*, $J_{11\beta,12\beta}$ = 4.8 Hz, $J_{11\beta,11\alpha}$ = 14.4 Hz, equatorial Hβ-11), 2.31 (1*H*, *dd*, $J_{11\alpha,12\beta}$ = 10.2 Hz, $J_{11\alpha,11\beta}$ = 14.4 Hz, axial Hα-11) and 1.33 (3H, *s*, 3H-20); all these assignments were confirmed by double resonance expts. EIMS (direct inlet) 75 eV, *m/z* (rel. int.): 338 $[M]^+$ (45), 323 (15), 310 (3), 265 (9), 241 (10), 227 (18), 183 (21), 170 (22), 169 (22), 167 (24), 165 (17), 155 (63), 153 (42), 141 (100), 129 (36), 128 (58), 127 (30), 105 (69), 95 (42), 94 (40), 91 (27), 81 (36), 77 (30), 69 (18), 65 (30), 63 (29), 53 (21),

51 (37). (Found: C, 70.84; H, 5.36. $C_{20}H_{18}O_5$ requires: C, 70.99; H, 5.36%.)

Acknowledgements—We thank Palermo Botanic Garden Office for the facilities given for the collection and botanical classification of the plant material, Miss M. D. Casado and Mrs. M. Plaza, Madrid, for recording the ^1H NMR and ^{13}C NMR spectra, and Mr. J. Prieto, Madrid, for elemental analyses. This work was supported in part by the National Research Council (CNR), Italy, and in part by the 'Comisión Asesora de Investigación Científica y Técnica', Spain.

REFERENCES

1. Savona, G., Paternostro, M., Piozzi, F., Hanson, J. R., Hitchcock, P. B. and Thomas, S. A. (1978) *J. Chem. Soc. Perkin Trans.* 1, 643.
2. Savona, G., Paternostro, M., Piozzi, F. and Hanson, J. R. (1979) *J. Chem. Soc. Perkin Trans.* 1, 533.
3. Savona, G. and Rodríguez, B. (1980) *An. Quim.* **76**, 187.
4. García-Alvarez, M. C., Savona, G. and Rodríguez, B. (1981) *Phytochemistry* **20**, 481.
5. Mukherjee, K. S. and Ghosh, P. K. (1978) *J. Ind. Chem. Soc.* **55**, 292 and 850.
6. Rogers, D., Unal, G. G., Williams, D. J., Ley, S. V., Sim, G. A., Joshi, B. S. and Ravindranath, K. R. (1979) *J. Chem. Soc. Chem. Commun.* 97.
7. Ito, K. and Furukawa, H. (1969) *J. Chem. Soc. Chem. Commun.* 653.
8. Jiménez, M., Moreno, E. D. and Díaz, E. (1979) *Rev. Latinoam. Quim.* **10**, 166.
9. Wagner, H., Seitz, R., Lotter, H. and Herz, W. (1978) *J. Org. Chem.* **43**, 3339.
10. Bruno, M., Savona, G., Pascual, C. and Rodríguez, B. (1981) *Phytochemistry* **20**, 2259.
11. De Alvarenga, M. A., Gottlieb, H. E., Gottlieb, O. R., Magalhaes, M. T. and Da Silva, V. O. (1978) *Phytochemistry* **17**, 1773.
12. Overton, K. H., Weir, N. G. and Wylie, A. (1966) *J. Chem. Soc. C* 1482.
13. Brehm, L., Hodder, O. J. R. and Halsall, T. G. (1971) *J. Chem. Soc. C* 2529.
14. Papanov, G. Y., Malakov, P. Y. and Bohlmann, F. (1981) *Phytochemistry* **20**, 170.
15. Brieskorn, C. H. and Stehle, T. (1973) *Chem. Ber.* **106**, 922.
16. Eggert, H., VanAntwerp, C. L., Bhacca, N. S. and Djerassi, C. (1976) *J. Org. Chem.* **41**, 71.
17. Crabbé, P. (1968) *Applications de la dispersion rotatoire optique et du dichroisme circulaire optique en Chimie Organique*, p. 511 and references therein. Gauthier-Villars, Paris.