Phytochemistry, Vol. 28, No. 2, pp. 567–570, 1989.
Printed in Great Britain.

0031–9422/89 $3.00 + 0.00
Pergamon Press plc.

ABIETANE TYPE DITERPENOIDS FROM *SALVIA FRUTICULOSA*. A REVISION OF THE STRUCTURE OF FRUTICULIN B*

LYDIA RODRÍGUEZ-HAHN,† BALDOMERO ESQUIVEL, CRISTINA SÁNCHEZ, LUIS ESTEBANES, JORGE CÁRDENAS, MANUEL SORIANO-GARCÍA, RUBÉN TOSCANO and T. P. RAMAMOORTHY‡

Instituto de Química, Universidad Nacional Autónoma de México, Circuito Exterior, Ciudad Universitaria, Coyoacán 04510, México, D.F; ‡Instituto de Biología, Universidad Nacional Autónoma de México, Circuito Exterior, Ciudad Universitaria, Coyoacán 04510, México, D.F.

(*Received in revised form* 13 *June* 1988)

Key Word Index—*Salvia fruticulosa*; Labiatae; *nor*- and *bisnor*-abietane diterpenoids; fruticulins.

Abstract—The structures of fruticulin A and its free phenol derivative, highly oxidized diterpene quinones isolated from *Salvia fruticulosa*, were determined by spectroscopic methods, chemical transformations and X-ray crystallographic analysis of one of them. The structure of fruticulin B is revised to a linear arrangement as a result of an X-ray diffraction analysis.

INTRODUCTION

The phytochemical studies of European and Asiatic *Salvia* species have led to the isolation of a number of diterpenes with an abietane skeleton [1] in contrast to the clerodane type diterpenoids isolated from the majority of the American *Salvia* species [2]. The American *Salvia* species have been classified in the subgenera *Leonia*, mostly found in North America, and *Calosphace*, to which belong most of the *Salvia* species found in Mexico, Central and South America [3, 4]. The subgenus *Calosphace* has been subdivided in 91 sections [3]. *Salvia fruticulosa* was classified in the section *Tomentellae* of the subgenus *Colosphace* (*Salvia*, Labiatae).

Some years ago the isolation and structural elucidation of icetexone, a rearranged abietane quinone isolated from *S. ballotaeflora* (section *Tomentellae*), was described [5]. Since then several diterpene quinones with rearranged abietane skeleton 6:7:6 have been isolated from plants of the *Labiatae* [6] and *Cupressaceae* [7] families.

In a previous communication [8] we described the structural elucidation of fruticulins A and B isolated from *S. fruticulosa*. The structure **6** proposed for fruticulin B has been revised to **7** based on X-ray diffraction analysis [9]. Herein we wish to describe in detail the spectroscopic data and chemical transformations which led to the structures proposed.

RESULTS AND DISCUSSION

Fruticulin A, (**1**), $C_{20}H_{20}O_4$ (*m/z*, 324 M^+), showed in the IR spectrum a hydrogen bonded hydroxyl group (3370 cm^{-1}) and a highly conjugated quinonoid system (1657, 1603, 1555 cm^{-1}) which was confirmed by the UV spectrum (see Experimental). The ^1H NMR spectrum of **1** indicated the presence of an isopropyl group attached to the benzoquinone ring (δ1.25, *d*, 6H, $J = 7$ Hz; and 3.35, *septet*, 1H, $J = 7$ Hz), an aromatic methyl group (δ2.35, *s*, 3H) a methoxy group (δ3.8. *s*. 3H) and two aromatic protons in a *meta*-relationship (δ6.97 and 6.82, 2*d*, 1H each, $J = 3$ Hz). A singlet observed at δ7.6 (exchangeable with D_2O) was assigned to the hydrogen bonded hydroxyl group and a singlet at δ8.1 to a vinylic proton on a highly conjugated double bond. A triplet at δ6.95 (1H, $J = 7$ Hz) was shown to be coupled to a doublet observed at δ3.1 ($J = 7$ Hz) by double resonance experiments. These signals were therefore assigned to H-7 and to the C-6 methylene.

When the ^1H NMR spectrum of **1** was run in C_6D_6 with a degassed sample, the vinylic and aromatic region of the spectrum were better resolved. The aromatic protons were observed at δ6.7 and 7.0 as broad doublets ($J = 3$ Hz). Irradiation of the aromatic methyl signal allowed us to assign the lower field doublet (δ7.0) to H-3 as it was transformed into a sharp doublet ($J = 3$ Hz).

An expansion of the δ6–8.5 region, after a resolution enhancement treatment of data (s.e. = −20), showed an additional small coupling ($J = 0.5$ Hz) of the vinylic H-7 (δ6.9), which was observed as a triplet of double. This signal was transformed to a triplet on irradiation at δ8.1 (H-20).

The ^{13}C NMR spectrum of fruticulin A (Table 1) is in agreement with the proposed structure **1**. The C-6 methylene was observed as a triplet at δ28.42.

Catalytic hydrogenation of fruticulin A gave the dihydroderivative **4** which showed in the ^1H NMR spectrum the C-20 methylene group as triplet ($J = 2$ Hz) at δ3.95; the C-6 and C-7 methylene groups appeared as a multiplet at δ2.9. In the ^{13}C NMR spectrum of **4** the three methylene groups were observed as triplets at δ28.73 and 27.34 (Table 1).

The structure **1** proposed for fruticulin A was confirmed by X-ray diffraction analysis [8]. It constitutes the first example of an abietanoid diterpene quinone or quinodimethide, with an aromatic A ring and a seven-membered B ring.

*Contribution No. 926 from Instituto de Química, UNAM.
†Author to whom correspondence should be addressed.

Table 1. ^{13}C NMR spectral data of compounds **1**, **4** and **5***

C	1	4	5
C-1	112.25 (*d*)	112.27 (*d*)	122.12 (*d*)
C-2	157.58 (*s*)	157.79 (*s*)	148.82 (*s*)
C-3	121.55 (*d*)	114.8 (*d*)	124.10 (*d*)
C-4	136.14 (*s*)	139.97 (*s*)	138.69 (*s*)
C-5	129.7 (*s*)	136.65 (*s*)	132.66 (*s*)
C-6	28.42 (*t*)	28.73 (t)	29.0 (*t*)
C-7	140.80 (*d*)	28.73 (*t*)	66.74 (*d*)
C-8	133.27 (*s*)†	130.9 (*s*)	128.64 (*s*)†
C-9	132.18 (*s*)†	130.9 (*s*)	129.65 (*s*)†
C-10	135.78 (*s*)	146.07 (*s*)	137.41 (*s*)
C-11	183.23 (*s*)	183.76 (*s*)	140.68 (*s*)
C-12	154.7 (*s*)	150.10 (*s*)	142.80 (*s*)
C-13	127.14 (*s*)	125.54 (*s*)	135.93 (*s*)
C-14	183.95 (*s*)	187.43 (*s*)	147.29 (*s*)
C-15	25.2 (*d*)	24.4 (*d*)	27.29 (*d*)
C-16	19.62 (*q*)	19.94 (*q*)	‡
C-17	19.62 (*q*)	19.94 (*q*)	‡
C-18	20.14 (*q*)	20.04 (*q*)	19.66 (*q*)
C-20	133.81 *d*)	27.34 (*t*)	69.83 (*d*)
–OMe	55.39 (*q*)	55.21 (*q*)	

*Run at 20 MHz using $CDCl_3$ as solvent and TMS as internal standard. SFORD multiplicities are in parenthesis.

†Values in any vertical column may be interchanged.

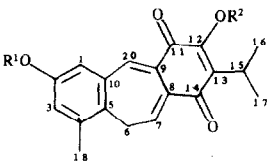
‡Overlapped with six methyl signals (OCOMe between δ 19.66 and 20.97. Six carbonyl signals are observed between δ 167.3 and 170.46 (OCOMe).

The most abundant diterpenoid product isolated from *S. fruticulosa* (0.01% dry weight) was demethyl fruticulin A (**2**), $C_{19}H_{18}O_4$ (*m/z*, 310, M^+). It showed in the IR spectrum a free phenolic band (3600 cm^{-1}), an hydrogen bonded hydroxyl group (3400 cm^{-1}) and the highly conjugated benzoquinone system (1657, 1603, 1556 cm^{-1}). In the ^1H NMR spectrum of **2** the absence of the methoxy group was evident, instead a broad signal at δ 5.25 which disappeared on addition of D_2O could be ascribed to the phenolic proton. The rest of the spectrum was very similar to that of fruticulin A (**1**). Attempts to transform **2** into the methyl derivative **1**, by treatment with ethereal diazomethane, were unsuccessful.

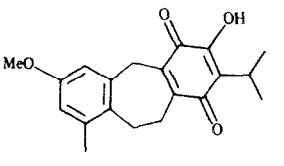
Treatment of **2** with acetic anhydride in the presence of sodium acetate at room temperature gave the diacetate derivative **3**, whose IR spectrum did not show hydroxyl absorption, the acetates carbonyl band was observed at 1769 cm^{-1}. In the ^1H NMR spectrum of **3**, two singlets at δ2.25 and 2.40 were ascribed to the acetate groups.

When **2** was treated with acetic anhydride in the presence of ethereal boron trifluoride the hexacetate derivative **5** was obtained. Its IR spectrum showed a strong band at 1774 cm^{-1} due to the aromatic acetate groups and a weaker one at 1729 cm^{-1} ascribed to the acetate groups bound to saturated carbon atoms. In the ^1H NMR spectrum of **5** in C_6D_6 solution at 70° an ABX system was observed due to the C-6 methylene (A: δ 3.98, 1H, J = 15 Hz; B: δ 3.50, 1H, J = 15 and 7 Hz), coupled to H-7 (X: δ 6.55. 1H. J = 7 Hz). Irradiation at δ 6.55 transformed the ABX system into an AB. It also showed five singlets at δ2.0 (6H), 1.45 (3H), 1.55 (3H), 1.72 (3H) and 1.78 (3H) ascribed to the six acetate methyl groups. The proposed structure **5** was confirmed by the ^{13}C NMR spectrum (Table 1) in which two doublets observed at δ 66.74 and 69.83 were assigned to C-7 and C-20 to which the secondary acetate groups are bound.

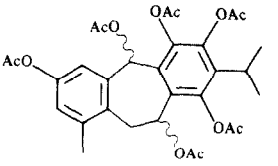
Fruticulin B was obtained in low yield (6×10^{-4}%) after repeated TLC of the crude fruticulin A, it has mp 173–174° and molecular formula $C_{19}H_{18}O_4$. The structure **6** previously proposed for it was based on spectroscopic evidence and biogenetic considerations. Its IR spectrum showed a hydrogen bonded hydroxyl group (3386 cm^{-1}) and a quinonoid absorption (1657, 1614, 1500 cm^{-1}). The UV spectrum revealed the presence of a highly conjugated quinone group (see Experimental). In the ^1H NMR spectrum the signals due to an isopropyl group attached to the benzoquinone ring appeared at δ 1.35 (*d*, 6H, J = 7 Hz) and 3.45 (*sept.*, 1H, J = 7 Hz). An aromatic methyl group was observed at δ2.70 (*s*, 3H) showing a paramagnetic displacement with respect to the aromatic methyl group in fruticulin A (**1**) which appeared at δ2.35. It also showed an aromatic methoxy group at δ 3.95 (*s*, 3H). A singlet at δ 7.50 (exchangeable with D_2O) was assigned to the hydrogen bonded hydroxyl group of the α-hydroxy *p*-benzoquinone moiety. The highly displaced singlets observed at δ 8.4 and 8.65 were ascribed to H-7 and H-14 of structure **6**. The paramagnetic shift of these protons as compared to those of taxodione (**8**), (δ 5.87 and 6.42) was attributed to the aromaticity of ring A and higher conjugation in the fruticulin B.



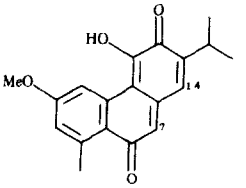
1 R^1 = Me, R^2 = H
2 R^1 = R^2 = H
3 R^1 = R^2 = Ac



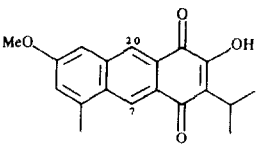
4



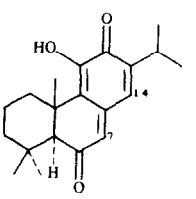
5



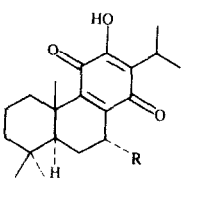
6



7



8



9 R = OAc
10 R = H

X-Ray crystallographic structural determination of fruticulin B revealed [9] a linear arrangement of the aromatic tricyclic skeleton represented by **7**. Structure **7** is the first to be reported of a natural 2-hydroxy1,4-anthracene quinone of abietanoid origin. It can be biogenetically derived from fruticulin A by oxidation of the C-6 methylene and ring B contraction. The highly displaced singlets observed at δ 8.4 and 8.65 in the ^{1}H NMR spectrum of fruiticulin B, can be unambiguously ascribed to H-7 and H-20 of structure **7** (the numbering in **7** follows biogenetic considerations). The 7α-acetoxy royleanone **9** was the only diterpenoid isolated from the roots of *S. fruticulosa*.

EXPERIMENTAL

Mps: uncorr. MS direct inlet at 70 eV. ^{1}H NMR were performed at 80 MHz, in $CDCl_3$ or C_6D_6 solns with TMS as int. standard. ^{13}C NMR were performed at 20 MHz in $CDCl_3$. Assignments of ^{13}C NMR were made with the aid of off-resonance, noise and gated decoupling experiments. Plant material was collected in Dec. 1983 and Sept. 1984 near Nochistlán (Oaxaca, México). Voucher specimens were deposited at the Herbarium of the Instituto de Biología, UNAM.

Isolation of diterpenoids from S. fruticulosa *Benth.* (Voucher MEXU-404012). Dried aerial parts (3 kg) of *S. fruticulosa* were extracted with Me_2CO (20 l) at room temp. for 1 week. The solvent was removed under red. pres. and the gummy residue obtained (100 g) chromatographed over silica gel (1.4 kg deactivated with 5% H_2O). Elution with petrol–EtOAc (19:1) afforded 125 mg of an orange solid. TLC in several solvent systems, revealed a two components mixture which was resolved by prep. TLC using C_6H_6 as eluent, 20 mg of fruticulin B and 80 mg of fruticulin A were obtained.

Fruticulin A (**1**), (0.0026% dry wt): mp 190–193° (CH_2Cl_2); UV λ_{max}^{MeOH} nm (ε): 215 (32000), 250 (10000), 278 (11500), 325 (16600), 420 (4216); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3672, 3371, 1657, 1603, 1555, 1467, 1440, 1394, 1300, 1275, 1178; ^{1}H NMR ($CDCl_3$) 80 MHz: δ 6.97 (*d*, J = 3 Hz, H-1), 6.82 (*d*, J = 3 Hz, H-3), 3.1 (*d*, J = 7 Hz, H-6), 6.95 (*t*, J = 7 Hz, H-7), 8.1 (*s*, H-20), 2.35 (*br s*, 3H-18), 7.6 (*s*, exchangeable with D_2O, –OH ring C), 3.35 (*sept*, J = 7 Hz, H-15), 1.25 (*d*, J = 7 Hz, 6H, Me-16, Me-17), 3.8 (*s*, –OMe); ^{13}C NMR see Table 1; MS *m/z* (rel. int.); 325 (20), 324 (100), 309 (30), 296 (15), 281 (20), 253 (10), 213 (75), 185 (20), 141 (20), 139 (15), 115 (10), 43 (10); 41 (10), 39 (10). $C_{20}H_{20}O_4$ requires M$^+$ at *m/z* 324.

Fruticulin B (**7**), (0.0006% dry wt): mp 173–174° (Me_2CO); UV λ_{max}^{MeOH} nm (ε): 210 (27500), 230 (43000), 300 (37000), 340 (17000), 400 (4300); IR $\nu_{max}^{CHCl_3}$ cm^{-1}; 3386, 1657, 1614, 1501, 1469, 1406, 1385, 1266, 1053; ^{1}H NMR ($CDCl_3$) 80 MHz: δ 7.1 (*br s*, overlapped signal for H-1 and H-3), 8.4 (*s*, H-7), 8.65 (*s*, H-20), 2.7 (*s*, 3H-18), 7.5 (*s*, exchangeable with D_2O, O<u>H</u> ring C), 3.45 (*sept*, J = 7 Hz, H-15), 1.35 (*d*, J = 7 Hz, 6H, Me-16, Me-17), 3.95 (*s*, –OMe); MS *m/z* (rel. int.): 311 (30), 310 (100), 295 (10), 267 (20), 253 (10), 239 (5), 199 (5), 165 (10),152 (10), 139 (10), 128 (5), 127 (10), 55 (5), 41 (10), 39 (10). $C_{19}H_{18}O_4$ requires M$^+$ at *m/z* 310.

Elution with petrol–EtOAc (8:2) led to the isolation of 300 mg of demethyl fruticulin (**2**) as a crystalline solid (0.01% dry wt): mp 200–203° (CH_2Cl_2): UV λ_{max}^{MeOH} nm (ε): 210 (31000), 250 (10200), 275 (12000), 325 (16500); IR $\nu_{max}^{CHCl_3}$: 3594, 3368, 1657, 1602, 1556; ^{1}H NMR ($CDCl_3$) 80 MHz: δ 6.81 (*d*, J = 3 Hz, H-1), 6.75 (*d*, J = 3 Hz, H-3), 3.1 (*d*, J = 7 H H-6), 6.92 (*t*, J = 7 Hz, H-7), 8.0 (*s*, H-20), 2.35 (*s*, 3H-18), 7.65 (*s*, exchangeable with D_2O, –O<u>H</u> ring C), 5.4 (*br s*, exchangeable with D_2O, –OH ring A), 3.35 (*sept*, J = 7 Hz, H-15), 1.25 (*d*, J = 7 Hz, 6H, Me-16, M-17); MS *m/z* (rel. int.): 311 (30), 300 (100), 295 (25), 282 (20), 267 (15), 239 (10), 200 (15), 199 (93), 171 (30), 170 (10), 152 (10), 141 (10), 139 (10), 115 (20), 43 (10), 41 (5), 39 (10). $C_{19}H_{18}O_4$ requires M$^+$ at *m/z* 310.

Catalytic hydrogenation of **1**. Fruticulin A (50 mg in EtOAc (5 ml) was hydrogenated using Pd/C (10%, 12 mg) as catalyst, during 2 hr. After usual work-up, product **4** was obtained. Mp 146–148°; UV λ_{max}^{MeOH} nm (ε): 205 (44000), 272 (12655). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3405, 1676, 1609, 1487, 1468, 1399, 1292, 1145; ^{1}H NMR ($CDCl_3$) 80 MHz: δ 6.6 (*br s*, overlapped signal for H-1, H-3), 2.95 (*m*, H-6), 2.9 (*m*, H-7), 3.95 (*t*, J = 2 Hz, H-20), 2.32 (*s*, 3H-18), 7.15 (*s*, exchangeable with D_2O, –OH ring C), 3.12 (*sept*, J = 7 Hz, H-15), 1.25 (*d*, J = 7 Hz, 6H, Me-16, Me-17), 3.75 (*s*, –OMe); ^{13}C NMR see table 1; MS *m/z* (rel. int.): 327 (20), 326 (100), 311 (20), 293 (15), 283 (15), 265 (10), 141 (20), 139 (15), 135 (10), 129 (10), 128 (20), 115 (20), 91 (20), 83 (20), 77 (15), 51 (20), 43 (20), 41 (20), 39 (20). $C_{20}H_{22}O_4$ requires M$^+$ at *m/z* 326.

Acetylation of demethyl fruticulin A with Ac_2O–AcONa. Demethyl fruticulin A (50 mg) was treated with Ac_2O (5 ml) and freshly dried AcONa (50 mg). The mixture was stirred for 8 hr at room temp. After usual work-up, the oily product **3** was obtained (76 mg). IR $\lambda_{max}^{CHCl_3}$ cm^{-1}: 1769, 1674, 1606, 1506. ^{1}H NMR ($CDCl_3$) 80 MHz: δ 7.1 (*br s*, overlapped signals for H-1 and H-3), 3.15 (*d*, J = 8 Hz, H-6), 6.92 (*t*, J = 8 Hz, H-7), 8.1 (*s*, H-20), 2.45 (*s*, 3H-18), 3.5 (*sept*, J = 7 Hz, H-15), 1.25 (*d*, J = 7. Hz, 6H, Me-16, Me-17), 2.4 and 2.3 (two acetate methyl groups).

Treatment of demethyl fruticulin A (**2**) *with* Ac_2O-BF_3–Et_2O. Compound **2** (100 mg) was treated with 2 ml of Ac_2O and 0.1 ml of $BF_3 \cdot Et_2O$. The reaction mixture was stirred for 30 min. Ice was added and the mixture was stirred for another hr. After the usual work-up, the gum obtained was separated by flash chromatography on silica gel using a petrol–EtOAc (7.5:1.5) mixture as eluent, yielding **3** as the minor product (10.5 mg). The more abundant product **5** (45 mg) was obtained as a crystalline compound: mp 258–260°; UV λ_{max}^{MeOH} nm (ε): 210 (27500), 230 (43000), 300 (37000), 340 (17000), 400 (4300); ^{1}H NMR C_6D_6 at (70°C): δ 7.15 (*d*, J = 3 Hz, H-1), 6.9 (*d*, J = 3 Hz, H-3), 3.5 (*dd*, J = 15 Hz and 6 Hz, H-6) 3.95 (*dd*, J = 15 and 1 Hz, H-6'), 6.55 (*dd*, J = 6 and 1 Hz, H-7), 7.5 (*br s*, H-20), 2.0 (*br s*, 3H-18), 3.1 (*sept*, J = 7 Hz, H-15), 1.25 (*d*, J = 7 Hz, Me-16, Me-17), 1.45–2.2 (six methyl signals –OCOMe), ^{13}C NMR see Table 1; MS *m/z* (rel. int.): 496 (4), 479 (3), 352 (100 base peak), 43 (47). $C_{31}H_{34}O_{12}$ requires M$^+$ at *m/z* 598 not observed.

Isolation of diterpenoid constituents from S. fruticulosa. (Voucher MEXU 404000). The roots and aerial parts of a second population of *S. fruticulosa* were analysed separately. Dried roots (2 kg) were extracted with Me_2CO (10 l) for 1 week. The solvent was removed under red. press. to yield 52 g of an extract, which was chromatographed over 500 g of silica gel (deactivated with 5% H_2O). Mixtures of petrol–EtOAc were used as solvent systems. Elution with petrol–EtOAc (19:1) afforded 480 mg of a yellow solid which was identified as 7α-acetoxy royleanone (**9**) by comparison of physical properties with literature data [10].

Catalytic hydrogenolysis of 7α-acetoxyroyleanone. (**9**). Compound **9** (50 mg) was hydrogenolysed in 10 ml of EtOAc, using Pd/C (12 mg, 10%) as catalyst. Usual work-up afforded 30 mg of a yellow crystalline product identified as royleanone **10** by comparison with literature data [10].

The aerial parts (leaves and stems) (8 kg) of this population of *S. fruticulosa* were extracted with Me_2CO (20 l) for one week. The gummy extract obtained (300 g) was chromatographed over silica gel (1.5 kg deactivated with 10% H_2O). Elution with mixtures of petrol–EtOAc lead to the isolation of fruticulin A (644 mg) and demethyl fruticulin A (800 mg). Fruticulin B was not isolated in this population.

Acknowledgements—The authors are indebted to Messrs R. Villena, M. Torres, H. Bojorquez, L. Velasco and R. Gaviño for

technical assistance. This work was supported in part by the Consejo Nacional de Ciencia y Tecnología México, (Project PCCBBNA 021142).

REFERENCES

1. Patudin, A., Romanowa, A., Sokolowa, W. S. and Pribylowa, G. (1974) *Planta Med.* **26**, 201.
2. Esquivel, B., Cárdenas, J., Ramamoorthy, T. P. and Rodríguez-Hahn, L. (1986) *Phytochemistry* **25**, 2381.
3. Epling, C. (1939) *Rep. Spec. Nov. Beih.* **110**, 1.
4. Ramamoorthy, T. P. (1984) *Plant Syst. Evol.* **146**, 141.
5. Watson, W., Taira, Z., Domínguez, X., González, H., Gutiérrez, M. and Aragón, R. (1976) *Tetrahedron Letters* **29**, 2501.
6. Kelecom, A. (1983) *Tetrahedron* **39**, 3603.
7. Hasegawa, Sh., Kojima, T. and Hirose, V. (1985) *Phytochemistry* **24**, 1545.
8. Rodríguez-Hahn, L., Esquivel, B., Sánchez, C., Cárdenas, J., Estebanes, L., Soriano-García, M., Toscano, R. and Ramamoorthy, T. P. (1986) *Tetrahedron Letters* **27**, 5459.
9. Soriano-García, M., Esquivel, B., Toscano, R., Estebanes, L. and Rodríguez-Hahn, L. (1988) *J. Cryst. Spect. Res.* (in press).
10. Edwards, O. E., Feniak, G. and Los, M. (1962) *Can J. Chem.* **40**, 1540.