*Phytochemistry*, Vol. 30, No. 6, pp. 1947–1950, 1991
Printed in Great Britain.

0031-9422/91 $3.00+0.00


# ACYCLIC DITERPENES AND SESQUITERPENE LACTONES FROM *MONTANOA TOMENTOSA* SUBSP. *TOMENTOSA*

LEOVIGILDO QUIJANO, FEDERICO GÓMEZ-G., EDMUNDO SIERRA-R. and TIRSO RIOS

Instituto de Química, Universidad Nacional Autónoma de México (UNAM), Circuito Exterior, Ciudad Universitaria, Coyoacán 04510, México, D.F.

(*Received* 25 *September* 1990)

**Key Word Index**—*Montanoa tomentosa* subsp. *tomentosa*; Asteraceae; Heliantheae; sesquiterpene lactones; acyclic diterpenes; geranylnerol derivatives.

**Abstract**—The aereal parts of *Montanoa tomentosa* subsp. *tomentosa* afforded, in addition to the known flavone salvigenin, and the sesquiterpene lactones zoapatanolides A, C, D and pumilin, two new acyclic diterpenes, zoapatols A and B as well as a new guaianolide, zoapatanolide F. The structures were established by spectroscopic methods and some chemical transformations.

## INTRODUCTION

Previous chemical studies of different populations of the Mexican plant 'zoapatle' (*Montanoa tomentosa* Cerv.) collected around México City, have demonstrated the presence of several sesquiterpene lactones [1, 2], in addition to the biologically active oxepane diterpenoids zoapatanol, tomexanthol, tomentol and their corresponding acyclic precursors [3, 4]. Here we describe the results of our study on *Montanoa tomentosa* subsp. *tomentosa* from Oaxaca, México. Besides widespread compounds and the known lactones zoapatanolide A (**11**), C (**12**), D (**13**) and pumilin (**14**) we isolated two new acyclic diterpenes, which we named zoapatol A (**1**) and B (**2**) as well as a new guaianolide, zoapatanolide F (**10**). The structures of the new compounds were established by some chemical transformations and spectral methods, mainly $^1$H NMR correlations with the previously established pre-zoapatanol (**8**) and pumilin (**14**).

## RESULTS AND DISCUSSION

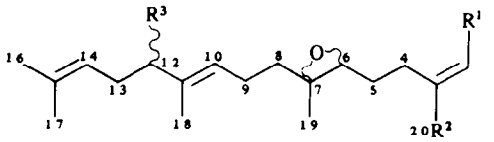
Zoapatol A (**1**), $C_{22}H_{36}O_5$, was a gummy acyclic diterpene characterized as a geranylnerol derivative containing hydroxyl groups and a saturated ester (IR bands at 3607 and 1730 cm$^{-1}$). The structure of the diterpene **1** could be deduced from the $^1$H NMR and mass spectral data by comparison with the spectral parameters of pre-zoapatanol (**8**) previously isolated from *Montanoa tomentosa* [4]. The spectral data suggested that zoapatol A (**1**) differs from **8** by the presence of: (a) an acetoxy group at C-1 instead of a hydroxyl group, (b) an extra double bond at C-10 and (c) a secondary hydroxyl group at C-12 instead of a keto group. The $^1$H NMR spectrum (Table 1) showed the expected differences from that of **8**. It showed a three proton sharp singlet at $\delta$2.04 in addition to the paramagnetic shift of the H-1 methylene doublet from $\delta$4.20 to 4.63. The secondary methyl group doublet was replaced by a vinyl methyl singlet at $\delta$1.62 and an extra vinyl proton triplet at $\delta$5.35 ($J$ = 7.0 Hz). Other significant differences resulted from the replacement of the C-12 keto function by a secondary hydroxyl group in **1**. Accordingly the spectrum of **1** did not show the signal of the methylene group $\alpha$ to the carbonyl function (H-13) at $\delta$3.12, but showed a new triplet at $\delta$3.95 ($J$ = 7.0 Hz) due to H-12. Low resolution EI mass spectrometry of the diterpene **1**, did not yield the molecular ion, but the CI mass spectrometry gave a [M + 1] ion ($m/z$ 381) corresponding to $C_{22}H_{36}O_5$ as well as the expected sequence of losses of water and acetic acid; $m/z$ 363 $[M-H_2O]^+$, 345 $[M-2H_2O]^+$, 303 $[M-H_2O-HOAc]^+$ and 285 $[M-2H_2O-HOAc]^+$.

Acetylation of **1** afforded a mixture of the triacetate **3** and the diacetate **4** which were separated by prep. TLC. The $^1$H NMR spectra of **3** and **4** showed minor but distinct differences. While the spectrum of **3** (Table 1) clearly indicated the presence of two extra sharp acetate peaks and the downfield shift of the H-12 and H-20 signals, the spectrum of **4** exhibited only one extra acetate peak and the downfield shift of the H-20 signal. The presence of the secondary hydroxyl group at C-12 in **4** was indicated by the nearly unchanged triplet at $\delta$3.95 as well as the IR absorption at 3595 cm$^{-1}$.

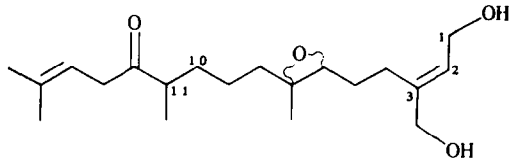
The stereochemistry at $\Delta^2$ in zoapatol A (**1**) was assigned to be *E* as in pre-zoapatanol (**8**) [4], based on the chemical shift of H-2 in **1**, **3**, and **4**. Partial oxidation of **1** with manganese dioxide gave the aldehyde **5**, which confirmed the *E*-configuration of the 2,3-double bond, as the chemical shift of the aldehyde proton on C-20 ($\delta$9.40) indicated a *cis*-relationship between the aldehyde function and the vinylic hydrogen [5]. The stereochemistry of the hydroxyl group at C-12 and the epoxy at C-6, C-7 remains open.

From more polar fractions, another acyclic diterpene, zoapatol B (**2**) was isolated. The structure of **2** was obviously closely related to that of **1**. The $^1$H NMR spectrum of **2** was similar to that of **1**, indicating that the

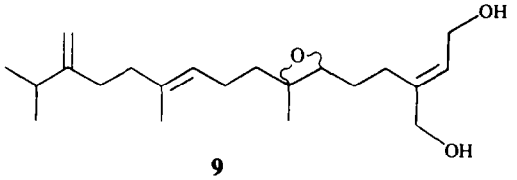
Contribution No. 1045 from Instituto de Química, UNAM. In commemoration of its 50th anniversary.



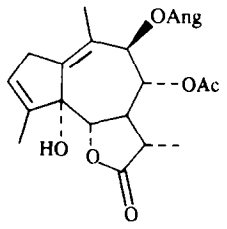
| | $R^1$ | $R^2$ | $R^3$ |
|---|---|---|---|
| **1** | $CH_2OAc$ | $CH_2OH$ | OH |
| **2** | $CH_2OH$ | $CH_2OH$ | H |
| **3** | $CH_2OAc$ | $CH_2OAc$ | OAc |
| **4** | $CH_2OAc$ | $CH_2OAc$ | OH |
| **5** | $CH_2OAc$ | CH=O | OH |
| **6** | $CH_2OAc$ | $CH_2OAc$ | H |
| **7** | CH=O | CH=O | H |



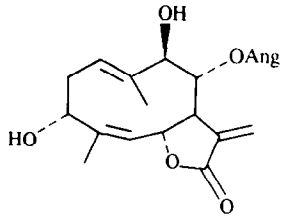
**8**



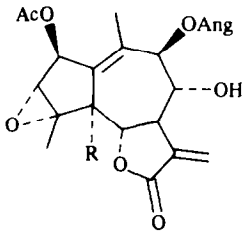
**9**



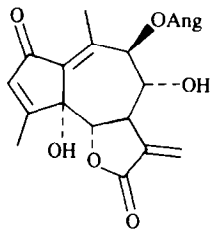
**10**



**11**



**12** R = OH
**13** R = H



**14**

difference resided in the absence of the acetoxy group at C-1 and the hydroxyl group at C-12. Accordingly the H-1 doublet now was shifted upfield at $\delta$4.20. The H-12 triplet was missing and the H-10 signal showed a small upfield shift due to the absence of the hydroxyl group at C-12. The diterpene **2** also showed no molecular ion in the IE mass spectrum. However, the CI mass spectrum showed a weak [M + 1] ion (*m/z* 323) in agreement with the molecular formula $C_{20}H_{34}O_3$ as well as strong peaks corresponding to the losses of water (*m/z* 305, 287 and 269).

Mild acetylation of **2** gave the corresponding diacetate **6**. The $^1$H NMR spectrum showed the presence of two acetate peaks at $\delta$2.05 and 2.08 and a downfield shift of the H-1 and H-20 signals. The stereochemistry of the 2,3-double bond again followed from the chemical shifts of H-1 and H-20 in the spectrum of the dialdehyde **7**, which were in agreement with the dialdehyde obtained from 20-hydroxygeranylnerol [6].

The presence of small singlets of $\delta$4.73 and 4.65 and a doublet at $\delta$1.04 ($J$ = 7.0 Hz) in the $^1$H NMR spectrum of **2** and a peak at *m/z* 335 in its CI mass spectrum, indicated the presence of small amounts of the homoditerpene **9** related with tomentol and pre-tomentol isolated before [4].

From the biogenetic point of view the isolation of zoapatols A (**1**) and B (**2**) is interesting because they could be considered as the earlier precursors of the oxepane diterpenoids present in 'zoapatle plant'. Zoapatol A (**1**), which might be derived from zoapatol B (**2**) by an allylic bio-hydroxylation at C-12, could be considered as the isomeric precursor of pre-zoapatanol (**8**) [4].

Zoapatanolide F (**10**), $C_{22}H_{28}O_7$ ([M]$^+$ at *m/z* 404), is an unstable sesquiterpene lactone, mp 182–184°, with an IR spectrum showing the presence of a tertiary hydroxyl group (3591, 1150 cm$^{-1}$), a $\gamma$-lactone (1775 cm$^{-1}$), a saturated ester (1740 cm$^{-1}$) and an $\alpha,\beta$-unsaturated ester (1719 cm$^{-1}$). The ester substituents were assigned to an acetate and an angelate group on the basis of diagnostic $^1$H NMR signals; a sharp three proton singlet at $\delta$2.01 for the acetate and one vinyl proton at $\delta$6.21 and the vinyl methyl signals at $\delta$1.95 and 2.05 for the angelate moiety, together with characteristic mass spectral peaks at *m/z* 43, 83 and 55.

The $^1$H NMR spectrum of lactone **9** did not show the typical signals of the exomethylene protons. Instead a three proton doublet at $\delta$1.25 (C-11 methyl) appeared. Upon irradiation of the C-11 methyl doublet at $\delta$1.25 ($J$ = 7.0 Hz), a doublet of quartets at $\delta$2.50 ($J$ = 7.0, 12.0 Hz,

Table 1. $^1$H NMR spectral data for diterpenes **1** and **2** and derivatives (80 MHz, $CDCl_3$, TMS as int. standard)

| H | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|
| 1 | 4.63 *d* (7)* | 4.20 | 4.63 | 4.61 | 4.91 | 4.62 | 10.32 |
| 2 | 5.64 *br t* (7)† | 5.75 | 5.64 | 5.63 | 6.50 | 5.64 | 6.53 *d* (7) |
| 6 | 2.73 *t* (7) | 2.75 *dd* (8, 5) | 2.70 *t* (7) | 2.70 | 2.70 *dd* (7, 6) | 2.70 *t* (7) | 2.72 *dd* (8, 6) |
| 10 | 5.35 *br t* (7) | 5.07 | 5.35 | 5.35 | 5.35 | 5.06 | 5.05 |
| 12 | 3.95 *t* (7) | — | 5.05 | 3.94 | 3.95 | — | — |
| 14 | 5.06 *br t* (7) | 5.07 | 4.95 | 5.06 | 5.07 | 5.05 | 5.05 |
| 16 | 1.70 *br s* | 1.69 | 1.66 | 1.70 | 1.71 | 1.66 | 1.67 |
| 17 | 1.62 *br s* | 1.60 | 1.60 | 1.63 | 1.65 | 1.60 | 1.61 |
| 18 | 1.62 *br s* | 1.60 | 1.60 | 1.63 | 1.65 | 1.60 | 1.61 |
| 19 | 1.25 *s* | 1.27 | 1.25 | 1.25 | 1.25 | 1.25 | 1.25 |
| 20 | 4.08 *br s* | 4.10 | 4.52 | 4.52 | 9.40 *s* | 4.52 *br s* | 9.64 *s* |
| OAc | 2.04 *s* | — | 2.01 | 2.05 | 2.10 | 2.05 | |
| | | | 2.05 *s* | 2.07 | | 2.08 | |
| | | | 2.08 *s* | | | | |

*Figures in parentheses are coupling constants or line separations in Hz.
†Multiplicities and coupling constants are not repeated if identical with those in previous column.

H-11) collapsed to a doublet ($J = 12.0$ Hz). The signal of the lactonic proton appeared as a doublet at $\delta$4.01 ($J = 10.5$ Hz, H-6) coupled with a signal at $\delta$3.20 (H-7) which in turn was also coupled with a triplet at $\delta$5.20 (H-8). The large couplings $J_{6,7} = 10.5$ Hz and $J_{7,8} = J_{8,9} = 11.0$ Hz indicated antiperiplanar arrangements of H-6, H-7, H-8 and H-9.

The $\alpha$-configuration of the C-11 methyl group followed from the coupling constant ($J_{7,11} = 12.0$ Hz) characteristic for 11$\beta$H,13-dihydrosesquiterpene lactones [7]. Finally the relative position of the angelate at C-9 as shown in structure **10** is solely based on the co-occurrence of the known zoapatanolides C (**12**), D (**13**) and pumilin (**14**). Therefore, the sites of attachment of the two ester groups in **10** remain tentative.

## EXPERIMENTAL

*Montanoa tomentosa* subsp. *tomentosa* was collected on 2 January 1986 in Oaxaca, México *ca* 2 km north of Tamazulapan on Hwy 190. Air-dried leaves (186 g), were ground and extracted with petrol, petrol–EtOAc (8:2) and EtOAc. After removing the solvent, the petrol–EtOAc extract (7 g) was fractionated by CC over silica gel (150 g) using $CH_2Cl_2$ and EtOAc, 8 frs of 250 ml each being collected. Frs **1** (552 mg) and **2** (474 mg) provided the widespread compounds taraxasteryl acetate, taraxasterol, kaurenoic acid and sitosterol. Fr. 3 (1.1 g) was rechromatographed over silica gel (80 g) using petrol–EtOAc mixts of increasing polarity, 38 frs of 50 ml each being collected. Frs 22–24 provided 10 mg of zoapatanolide F (**10**), frs 25–29 gave 22 mg of 5-hydroxy-6,7,4′-trimethoxyflavone, mp 190–192° [8] and fr. 35 gave 7 mg of zoapatanolide D (**13**) [2]. Frs 4 and 5 were combined (813 mg) and rechromatographed over silica gel as described above. Frs 10–14 gave further amounts of **13** (120 mg). Prep. TLC of the mother liquor provided 35 mg of zoapatanolide C (**12**) [4]. Fr. 6 (2.28 g) was rechromatographed over silica gel (80 g) using petrol–EtOAc mixts as eluants. Frs 20–23 yielded 50 mg of pumilin (**14**) [2], fr. 31 gave 40 mg of zoapatanolide A (**11**) [1] and fr. 34 provided 50 mg of zoapatol A (**1**). Rechromatography of fr. 8 gave 35 mg of zoapatol B (**2**).

*Zoapatol A* (**1**). $C_{22}H_{36}O_5$, gum; IR $\nu_{max}^{CHCl_3}$ cm$^{-1}$: 3608, 1730, 1450, 1379; EIMS (probe) 70 eV *m/z* (rel. int.): 311 $[M-C_5H_9]^+$ (0.5), 293 $[M-C_5H_9-H_2O]^+$ (1.1), 251 $[M-C_5H_9-HOAc]^+$ (11.5), 233 $[M-C_5H_9-HOAc-H_2O]^+$ (6.1), 215 $[M-C_5H_9-HOAc-2H_2O]^+$ (3.9), 97 $[C_6H_9O]$ (100.0), 43 $[C_2H_2O]^+$ (94.7); CIMS ($CH_4$) *m/z* (rel. int.): 381 $[M+1, C_{22}H_{36}O_5]^+$ (0.5), 363 $[M+1-H_2O]^+$ (20.7), 345 $[M+1-2H_2O]^+$ (26.8), 303 $[M+1-H_2O-HOAc]^+$ (93.7), 285 $[M+1-2H_2O-HOAc]^+$ (43.9), 149 $[C_{11}H_{17}]^+$ (100.0).

*Zoapatol B* (**2**). $C_{20}H_{34}O_3$, gum; IR $\nu_{max}^{CHCl_3}$ cm$^{-1}$: 3611, 3441, 1670, 1639, 1602, 1453, 1383; EIMS (probe) 70 eV *m/z* (rel. int.): 304 $[M-H_2O]^+$ (0.2), 123 (19.5), 109 (30.4), 81 (58.8), 69 (96.8), 43 (83.2), 41 (100.0); CIMS ($CH_4$) *m/z* (rel. int.): 323 $[M+1, C_{22}H_{34}O_3]^+$ (3.7), 305 $[M+1-H_2O]^+$ (80.5), 287 $[M+1-2H_2O]^+$ (41.5), 269 $[M+1-3H_2O]^+$ (31.2), 125 (62.1), 123 (67.0), 109 (100.0).

*Zoapatanolide F* (**10**). $C_{22}H_{28}O_7$, crystals, mp 182–184° ($Et_2O$); UV $\lambda_{max}^{MeOH}$ nm ($\varepsilon$): 210 (14950); IR $\nu_{max}^{CHCl_3}$ cm$^{-1}$: 3580, 1775, 1740, 1715; EIMS (probe) 70 eV *m/z* (rel. int.): 404 [M, $C_{22}H_{28}O_7]^+$ (3.1), 362 $[M-C_2H_2O]^+$ (2.4), 344 $[M-HOAc]^+$ (2.4), 304 $[M-C_5H_8O_2]^+$ (1.4), 147 (10.9), 83 (100.0), 55 (27.6), 43 (12.6); $^1$H NMR (80 MHz, $CDCl_3$): $\delta$1.25 (3H, *d*, $J = 7.0$ Hz, H-13), 1.65 (3H, *br s*, H-14), 1.87 (3H, *br s*, H-15), 1.95 (3H, *m*, H-5′), 2.05 (3H, *br d*, $J = 7.0$ Hz, H-4′), 2.01 (3H, *s*, OAc), 2.50 (*dq*, $J = 12.0$ and 7.0 Hz, H-11), 2.96 (2H, *br s*, H-2), 3.20 (*ddd*, $J = 12.0$ Hz and 10.5 Hz, H-7), 4.01 (*d*, $J = 10.5$ Hz, H-6), 5.20 (*t*, $J = 11.0$ Hz, H-8), 5.65 (*br s*, H-3), 6.21 (*br q*, $J = 7.0$ Hz, H-3′), 6.25 (*br d*, $J = 11.0$ Hz, H-9).

*Preparation of triacetate* **3** *and diacetate* **4**. Acetylation of **1** (20 mg) in $Ac_2O$ (0.5 ml) and pyridine (0.2 ml), followed by usual work-up gave after prep. TLC (petrol–EtOAc, 6:4) gave 3 mg of **3** and 4 mg of **4**. Triacetate **3**. gum; IR $\nu_{max}^{CHCl_3}$ cm$^{-1}$: 1732, 1452, 1372; EIMS (probe) 70 eV *m/z* (rel. int.): 464 $[M]^+$ (not observed), 404 $[M-HOAc]^+$ (0.2), 395 $[M-C_5H_9]^+$ (0.3), 344 $[M-2HOAc]^+$ (0.2), 335 $[M-C_5H_9-HOAc]^+$ (0.9), 293 $[M-C_5H_9-C_2H_2O-HOAc]^+$ (0.6), 275 $[M-C_5H_9-2HOAc]^+$ (0.6), 233 $[M-C_5H_9-C_2H_2O-2HOAc]^+$ (1.8), 215 $[M-C_5H_9-3HOAc]^+$ (1.6), 43 (100.0); CIMS ($CH_4$) *m/z* (rel. int.): 465 $[M+1, C_{26}H_{40}O_7]^+$ (0.7), 405 $[M+1-HOAc]^+$ (30.0), 345 $[M+1-2HOAc]^+$ (100.0), 285 $[M+1-3HOAc]^+$ (53.9), 267 $[M+1-3HOAc-H_2O]^+$ (33.5), 149 (78.8).

*Diacetate* **4**. Gum, IR $\nu_{max}^{CHCl_3}$ cm$^{-1}$: 3595, 1735, 1453, 1375; EIMS (probe) 70 eV *m/z* (rel. int.): 422 $[M]^+$ (not observed), 83 (21.9), 69 (30.4), 55 (31.0), 43 (100.0); CIMS ($CH_4$) *m/z* (rel. int.):

405 $[M+1-H_2O]^+$ (22.5), 345 $[M+1-H_2O-HOAc]^+$ (100.0), 285 $[M+1-H_2O-2HOAc]^+$ (63.4), 267 $[M+1-2H_2O-2HOAc]^+$ (43.8), 149 (76.3).

*Aldehyde* **5**. A 30 mg sample of **1** in 50 ml of $CH_2Cl_2$ was oxidized with $MnO_2$ (400 mg) for 5 hr at room temp. Usual work-up and TLC purification (petrol–EtOAc, 1:1) gave 8 mg of **5**. UV $\lambda_{max}^{MeOH}$ nm (ε): 222 (7092); IR $\nu_{max}^{CHCl_3}$ $cm^{-1}$: 3604, 1741, 1690, 1450, 1376; EIMS (probe) 70 eV *m/z* (rel. int.); 378 $[M]^+$ (not observed), 360 $[M-H_2O]^+$ (0.3), 309 $[M-C_5H_9]^+$ (4.8), 249 $[M-C_5H_9-HOAc]^+$ (12.2), 231 $[M-C_5H_9-HOAc-H_2O]^+$ (7.3), 97 (67.0), 81 (41.4), 69 (45.8), 55 (46.3), 43 (100.0); CIMS ($CH_4$) *m/z* (rel. int.): 379 $[M+1, C_{22}H_{34}O_5]^+$ (17.2), 361 $[M+1-H_2O]^+$ (100.0), 343 $[M+1-2H_2O]^+$ (52.8), 319 $[M+1-HOAc]^+$ (21.9), 301 $[M+1-HOAc-H_2O]^+$ (37.3), 283 $[M+1-HOAc-2H_2O]^+$ (36.5).

*Diacetate* **6**. Acetylation of **2** (25 mg) in $Ac_2O$–pyridine under standard work-up conditions gave 9 mg of **6**, gum, IR $\nu_{max}^{CHCl_3}$ $cm^{-1}$: 1736, 1460, 1375; EIMS (probe) 70 eV *m/z* (rel. int.): 406 $[M]^+$ (not observed), 346 $[M-HOAc]^+$ (0.2), 286 $[M-2HOAc]^+$ (0.2), 268 $[M-2HOAc-H_2O]^+$ (0.2), 109 (52.2), 81 (40.3), 69 (67.0), 43 (100.0); CIMS ($CH_4$) *m/z* (rel. int.): 407 $[M+1]^+$ (not observed), 347 $[M+1-HOAc]^+$ (45.3), 329 $[M+1-HOAc-H_2O]^+$ (19.5), 287 $[M+1-2HOAc]^+$ (45.8), 269 $[M+1-2HOAc-H_2O]^+$ (84.9), 109 (100.0).

*Dialdehyde* **7**. Oxidation of 10 mg of **2** with $MnO_2$ gave the dialdehyde **7**, gum IR $\nu_{max}^{CHCl_3}$ $cm^{-1}$: 1685, 1630, 1451, 1380; EIMS (probe) 70 eV *m/z* (rel. int.): 318 $[M, C_{26}H_{30}O_3]^+$ (0.2), 81 (67.0), 69 (100.0), 43 (67.0).

*Acknowledgements*—The authors wish to thank Dr Alfredo Ortega for plant collection, Mr Alejandro Cisneros for plant identification and Messrs J. Cárdenas, R. Gaviño, M. Torres and L. Velasco for technical assistance.

## REFERENCES

1. Quijano, L., Calderón, J. S., Gõmez-G, F. and Rios, T. (1982) *Phytochemistry* **21**, 2041.
2. Quijano, L., Gómez-G., F., Calderón, J. S., López-P., J. and Rios, T. (1984) *Phytochemistry* **23**, 125.
3. Quijano, L., Calderón, J. S., Gómez-G., F., Rosario-M., V. and Rios, T. (1985) *Phytochemistry* **24**, 2337.
4. Quijano, L., Calderón, J. S., Gómez-G., F., Rosario-M., V. and Rios, T. (1985) *Phytochemistry* **24**, 2741.
5. Chan, J. C., Jewell, R. A., Nutting, W. H. and Rapaport, H. (1968) *J. Org. Chem.* **33**, 3382.
6. Bohlmann, F., Ziesche, J., Robinson, H. and King, R. M. (1981) *Phytochemistry* **20**, 1146.
7. Narayanan, C. R. and Venkatasubramanian, N. K. (1968) *J. Org. Chem.* **33**, 3156.
8. Herz, W. and Govindan, S. V. (1980) *J. Org. Chem.* **45**, 1113.