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SESQUITERPENE LACTONES OF *ARTEMISIA KLOTZCHIANA**

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Key Word Index—*Artemisia klotzchiana*; Compositae; sesquiterpene lactones, ridentin; hanphyllin; chloroklotzchin; jaceosidin; sudatichin.

Abstract—Six sesquiterpene lactones and two flavones were isolated and characterized from *Artemisia klotzchiana*. These included chrysartemin A, matricarin, desacetyl matricarin, ridentin, hanphyllin, jaceosidin, sudatichin and the new guaianolide chloroklotzchin, which is a halogenated lactone. The X-ray structure of hanphyllin is reported.

INTRODUCTION

The genus *Artemisia* is the largest and most widely distributed in the tribe Anthemideae of the Asteraceae. As with most of the genera belonging to this tribe, the sesquiterpene lactones are mainly eudesmanolides, guaianolides and germacranolides. The *Artemisia* genus have been subdivided into four different sections; of these, the subgenus *Abrotanum* produce the most structurally diversified and biosynthetically advanced sesquiterpene lactones. In turn, the lactones in species from the subsection *Vulgares*, which comprise most of the North American species of the section *Abrotanum*, are biosynthetically and structurally similar with some compounds (matricarin **1**, desacetyl matricarin **2**, arglanin **9**) occurring in several species suggesting close phylogenetic relationships for these taxa [1].

Following our studies of Mexican *Artemisia* species [2–4], a new collection of *A. klotzchiana* Begs. was analysed resulting in the isolation and characterization of three additional sesquiterpene lactones, as well the two flavones sudachitin **7** and jaceosidin **8**. Two of the lactones were identified as hanphyllin **4** and ridentin **5**. The third one, chloroklotzchin **6**, is a new natural product.

RESULTS AND DISCUSSION

Air dried aerial parts of *A. klotzchiana* were extracted with dichloromethane–methanol (3:1). The extract was chromatographed on a silica gel column following standard procedures. Successive rechromatography of the initial fractions afforded six crystalline sesquiterpene lactones (**1**–**6**) and two flavones (**7** and **8**).

Lactone **4** was identified as hanphyllin by comparison of its spectral data with those previously reported [5] and by X-ray analysis. Hanphyllin crystallizes in the orthorhombic space group $P_{2_1 2_1 2_1}$ and possessed unit cell constants of $a = 10.2163$, $b = 11.4145$, $c = 11.5320$ Å with $Z = 4$. The perspective view of hanphyllin is represented by Fig. 1. The structure shows that the cyclodecadiene ring adopts a chair like crown conformation similar to that of costunolide [6]. The α-methylene-γ-lactone is *trans* fused at C-6 and C-7, and the C-3 hydroxyl group is β-oriented within the cyclodecadiene framework. Hanphyllin was previously isolated from *Artemisia ashrbajevii* Winki [5].

Lactone **5** was isolated as a crystalline powder. Its UV, IR, mass, ^{13}C NMR and ^{1}H NMR spectra were identical to those of ridentin which has been reported as a constituent of other species of *Artemisia* [7–9].

Lactone **6** had the composition $C_{15}H_{17}O_5Cl$ (elemental analysis and mass spectrometry), mp 230°. The IR spectrum showed hydroxyl group absorptions at 3500 and 3425 cm^{-1}, and bands at 1755 and 1630 cm^{-1} corresponding to an exocyclic methylene conjugated with a γ-lactone.

The ^{1}H and ^{13}C NMR data (Tables 1 and 2) were very similar to those of chrysartemin A (**3**) and chrysartemin B [2, 9–11], and confirmed the presence of the α-methylene-γ-lactone [^{1}H NMR (CDCl$_3$): pair of doublets at δ5.44 (1H, $J = 3$ Hz) and 6.17 (1H, $J = 3$ Hz); ^{13}C NMR: signals at δ169.8 (*s*), 140.9 (*s*), 118.7 (*t*) and 62.51 (*d*)]. In the ^{1}H NMR spectrum a typical ABC pattern was observed for the protons of C-6 (H_A), C-5 (H_B) and C-7 (H_C) H_A appeared as a doublet of doublets ($J_{AB} = 11$ Hz, $J_{AC} = 9.5$ Hz) centred at δ4.36, H_B as a doublet ($J_{AB} = 11$ Hz) at δ2.80 and H_C as a multiplet centred at δ3.55. The coupling among these protons suggested a *trans* fused C-6/C-7 lactone and these assignments were supported by decoupling experiments in the DMSO-d_6 spectrum: irradiation of the δ4.37 signal collapsed the H-5 absorption (δ2.66) to a singlet; conversely, irradiation of H-5 simplified the H-6 doublet of doublets to a well defined doublet ($J = 9.5$ Hz); irradiation of the δ3.57 region, where H-7 resonated, collapsed the doublets of the exocyclic methylene to singlets. Finally, irradiation of H-6 simplified the multiplet adscribed to H-7. The pair of well defined doublets at δ3.84 (1H, $J = 2$ Hz) and 4.07 (1H, $J = 2$ Hz) in the ^{1}H NMR, the latter shifting to δ5.06 upon treatment with TAI, as well as the signals at δ79.1 (*d*), 65.4 (*d*) and 72.5 (*s*) in the ^{13}C NMR, suggested the presence of a C-2 hydroxy group and a C-3/C-4 epoxide ring respectively. The deshielding effect on H-6, which compared very well with that on the similar proton in chrysartemin B, was consistent, not only with a β-oriented epoxide function at

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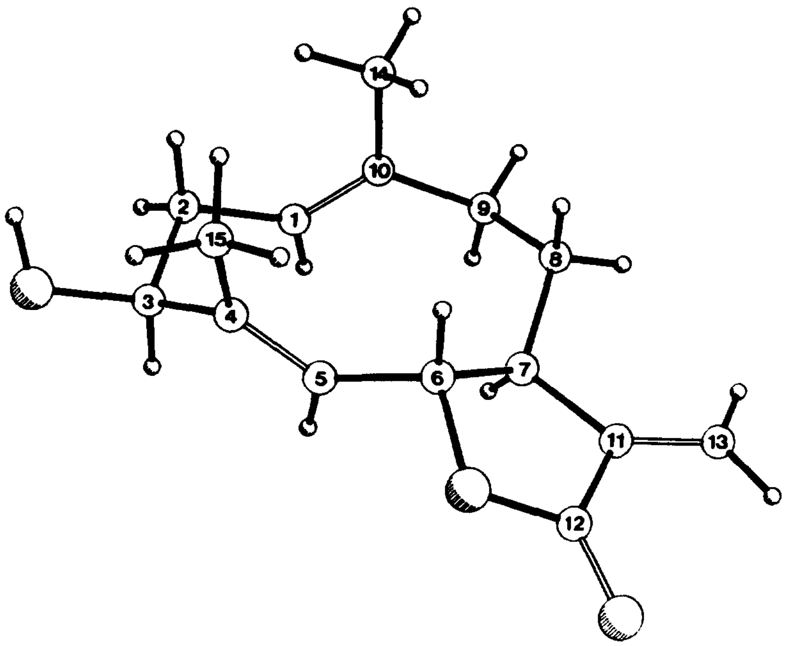


Fig. 1 Perspective view of hanphyllin

Table 1. ^{1}H NMR data for compound **6** (80 MHz, $CDCl_3$ or DMSO-d_6, TMS as internal standard)

H	$CDCl_3$	$CDCl_3$ + TAI	DMSO-d_6
2	4.02 *d*	5 06 *d*	4.15 *d*
	(2)	(2)	(2)
3	3.84 *d*	3 80 *d*	3.82 *d*
	(2)	(2)	(2)
5	2.80 *d*	2 86 *d*	2 66 *d*
	(11)	(11)	(11)
6	4.36 *dd*	4 71 *dd*	4 37 *dd*
	(11, 9 5)	(11, 9.5)	(11, 9.5)
7	3.55 *m*	3.25 *m*	3.57 *m*
13	5.44 *d*	5 5 *d*	5.47 *d*
	(3)	(3)	(3)
13′	6.17 *d*	6.17 *d*	5 97 *d*
	(3)	(3)	(3)
14	1 49 *s**	1.99 *s**	1.04 *s**
15	1.57 *d**	1.55 *s**	1.40 *d**
	1.8		1.8
OH†	3.17	—	4.03
OH†		—	
	—	8 36 *s* (*br*) 8 49 *s* (*br*)	—

Figures in parentheses are coupling constants in Hz
*Intensity equal to three protons.
†Disappeared after equilibration with D_2O.

Table 2. ^{13}C NMR spectra of compound **6** (20 MHz DMSO-d_6, TMS as internal standard)

C	δ
1	78.53 *s*
2	79.09 *d*
3	65.38 *d*
4	72.52 *s*
5	49.99 *d*
6	62.51 *d*
7	42.78 *d*
8	22.54 *t*
9	33.74 *t*
10	70 19 *s*
11	140.90 *s*
12	169.78 *s*
13	118.68 *t*
14	27 00 *q*
15	25.30 *q*

Signals were assigned by means of off resonance decoupled spectra and by using model compounds previously reported [9, 25]

C-3/C-4, but also with a β-oriented hydroxyl group at C-2 [11, 12]. In agreement with the β-orientation of the C-2 hydroxyl was the downfield shift (δ 1.99) observed for the protons of the C-14 methyl group and the absence of the β acyl effect [13] on H-3 in the ^{1}H NMR spectrum upon treatment with TAI.

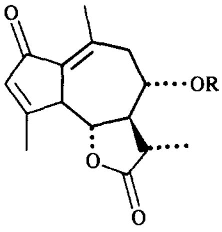
The low field of H-7 (δ 3.55 in $CDCl_3$ and δ 3.57 in DMSO-d_6), as well as the upfield shift of this proton in passing to the dicarbamate (δ 3.25) indicated that H-7 was in close proximity with the tertiary hydroxyl group at C-10 [14, 15]. Consistent with a tertiary hydroxyl group at C-10, was the absence of a proton geminal to the carbamate, other than that of H-2 in the ^{1}H NMR of the TAI derivative.

The chemical shift observed for H-5 was a little downfield compared with that of similar guaianolides, and was in agreement with the chlorine atom being α-oriented at C-10. In this position it eclipsed the H-5 proton and deshielded it from the normal position [11]. Also it was consistent with the downfield shift observed for the C-15 methyl group in the ^{13}C NMR, which could be attributed to a δ *syn* axial effect between a C-1 substituent and the C-15 methyl group [14]. Therefore, the most probable structure of the new halogenated lactone was **6**.

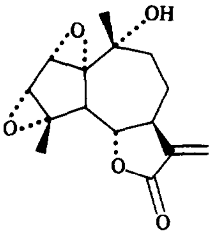
Flavone **8** showed similar spectral properties (UV, IR, ^{1}H NMR and MS) to those previously reported for jaceosidin. Furthermore, the physical constants as well as the spectral properties of a triacetate prepared by acetylation of **8** with pyridine–acetic anhydride were identical to those already published [16–18].

Flavone **7** was identified as sudachitine by comparison of the spectral characteristics and physical data of **7** and its methylated derivative with those described in the literature [19–21].

From the biogenetic point of view, the co-occurrence of lactones **1–6** might be of interest. The possibility that the Cl atom in **6** was introduced during the extraction process cannot be discounted. However, it is a well documented fact that chloro sesquiterpene lactones are indeed naturally occurring products [22, 23]. It would be reasonable to envisage chrysartemin B as the possible precursor of **6**, although it was not isolated in the present work. This last assumption is supported by the coexistence, in many instances, of such epoxides with their respective chlorhydrines [24]. It is of interest to note that the presence of a



1 R = H
2 R = Ac



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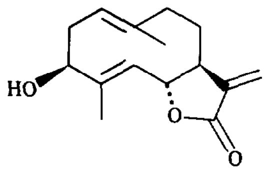

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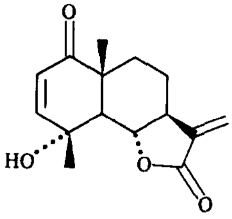

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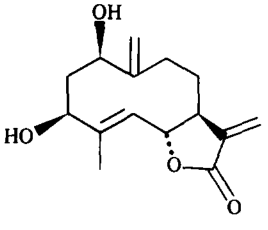

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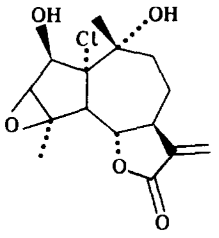

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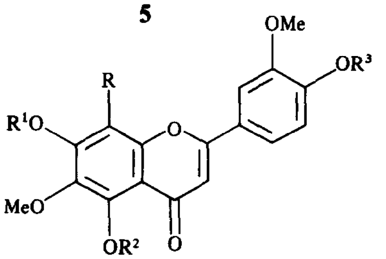

7 R = R^1 = R^2 = R^3 = H
10 R = H, R^1 = R^2 = R^3 = Ac
8 R = OMe, R^1 = R^2 = R^3 = H

halogenated sesquiterpene lactone has no precedent in the *Artemisia* genus.

EXPERIMENTAL

Mps· uncorr; elementary analysis of **5** was performed at Mic. Anal, Tucson, Arizona; MS: 70 eV.

Plant material. A. klotzchiana was collected *ca* 4 km SSW of Pachuca, Hidalgo, Hwy 45, in August 1983. Reference specimens are deposited in the National Herbarium, Instituto de Biología de la U.N.A.M. (voucher G. Delgado 00151).

Extraction Dried and shredded aerial parts of the plant (7 kg) were extracted with hexane at room temp. The defatted material was then extracted with CH_2Cl_2–MeOH (3:1) to yield 355 g of extract. 250 g of the extract was resolved by CC over silica gel (3.1 kg) using a C_6H_6–EtOAc gradient elution system, one litre fractions being collected.

Isolation of hanphyllin (**4**) *and sudachitin* (**7**). Fractions 101–103 (1.5 g), eluted with C_6H_6–EtOAc (3:1), from the original column were rechromatographed on silica gel (66 g), starting elution with C_6H_6–EtOAc (4:1) and then with increasing amounts of EtOAc: fractions 29–34 eluted with C_6H_6–EtOAc (4:1), when triturated with Me_2CO–*iso*-Pr_2O, gave 245 mg **4** (0.0049 % dry wt) mp 180° (lit. mp 167° [4]). IR ν_{max}^{KBr} cm^{-1}: 3480, 1745, 1280, 1250, 1145, 1060, 960, 940; ^{1}H NMR ($CDCl_3$, 80 MHz): δ1 45 (*d*, 3H, $J = 2$ Hz, H-14), 1.72 (*d*, 3H, $J \approx 2$ Hz, H-15), 4.26 (*dd*, 3H, $J = 9$, 6 Hz, H-3), 4.55 (*q*, 1H, $J = 10$ Hz, H-6), 5.57 (*d*, 1H, $J = 3$ Hz, H-13a), 6.26 (*d*, 1H, $J = 3$ Hz, H-13b); EIMS *m/z* (rel. int.): 248 [M$^+$, $C_{15}H_{20}O_3$] (26.7), 230 (4.8), 178 (14 6), 175 (19.5), 91 (56.9), 81 (70.0), 53 (100), 41 (78); X-ray analysis was performed on a Nicolet R 3 m/E crystallographic system. A single crystal of hanphyllin was obtained by recrystallization from Me_2CO–*iso*-Pr_2O The size of the crystal used for data collection was 3 × 22 × 44 mm, $\mu = 0.78$ cm^{-1}. A total of 1044 independent reflections were measured for $2\theta < 45$, of which 908 were considered. The structure was solved by direct methods and was refined by bloqued cascade matrix least square methods In the final refinement, anisotropic thermal parameters were used for the non-hydrogen atoms and isotropic temperatures were used for the hydrogen atoms. The final discrepancy indexes are $R = 0.0468$ and $R_w = 0.0494$ for the observed reflections Crystallographic data have been deposited with the Cambridge Crystallographic Data Centre. From fractions 35–39, also eluted with C_6H_6–EtOAc (4:1), were obtained 75 mg (0.0021 % of the dry wt) of a crystalline yellow powder, mp 240° (lit mp 239 5–240.5° [19], 239–241° [21]) IR ν_{max}^{KBr} cm^{-1}: 3462, 3414, 3385, 1652, 1603, 1513, 1471, 1362, 1290, 1212, 809; ^{1}H NMR ($CDCl_3$, 80 MHz): δ3.85 (*s*, 3H, OMe), 3.96 (*s*, 3H, OMe), 4.04 (*s*, 3H, OMe), 6.12 (*s* (*br*), OH), 6.53 (*s*, 1H, H-3), 7.00 (*d*, 1H, $J = 8$ Hz, H-5′), 7 63 (*dd*, 2H, $J = 8$, 2 Hz, H-6′), 7.65 (*d*, 1H, $J = 2$ Hz, H-2′), 11.35 (*s*, 1H, OH); UV λ_{max}^{MeOH} nm (ε). 281 (20 000), 345 (21 800), 264 (13 100), 307 (13 500).

Methylation of sudachitin. A soln of 15 mg **7** in 2 ml MeOH were treated with an excess of CH_2N_2 in Et_2O at room temp The mixture was left overnight, then evaporated The crystalline residue was recrystallized once from, MeOH to afford 12 mg **9**, mp 145° (lit. mp 143–144° [19]). IR ν_{max}^{KBr} cm^{-1}: 1656, 1594, 1514, 1460, 1358, 1265, 1219, 1147, 805; ^{1}H NMR ($CDCl_3$, 80 MHz) δ3.85 (*s*, 3H, OMe), 3 90 (*s*, 3H, OMe), 3 95 (*s*, 9H, OMe), 6.45 (*s*, 1H, H-3), 6.96 (*d*, 1H, $J = 8$ Hz, H-5′), 7 70 (*dd*, 1H, $J = 8.2$ Hz, H-2′), 7.65 (*d*, 1H, $J = 2$ Hz, H-6′), 11 15 (*s*, 1H, OH-5).

Isolation of jaceosidin (**8**) *and chloroklotzchin* (**6**). From fractions 102–104 eluted with C_6H_6–EtOAc (3:1) a yellow powder was separated and recrystallized first from Me_2CO and then from C_6H_6 to yield 1.0583 g (0.0214 % of the dry wt) of **8**, mp 230° (lit. mp 224–226° [17], 227–228° [16]). IR ν_{max}^{KBr} cm^{-1}: 3400, 1655, 1615, 1605, 1570, 1455, 1415, 1365, 1265, 1155; ^{1}H NMR ($CDCl_3$, 80 MHz): δ4.0 (*s*, 3H, OMe), 4 04 (*s*, 3H, OMe), 5.97 (*s* (*br*), 1H, OH), 6 45 (*s* (*br*), 1H, OH), 6.55 (*s*, 1H, H-3), 6 57 (*s*, 1H, H-8), 7 0 (*d*, 1H, $J = 8$ Hz, H-5′), 7.35 (*dd*, 1H, $J = 8$, 2 Hz, H-6′), 7.50 (*d*, 1H, $J = 2$ Hz, H-2′), 11 0 (*s*, 1H, OH-5); EIMS *m/z* (rel int)· 330 [M]$^+$ (100), 315 (51.21), 312 (83.2), 287 (93.7), 177 (21 9), 140 (34.1), 69 (98.6); UV λ_{max}^{MeOH} nm (ε): 213 (12 412), 273 (5340), 345 (8325). The mother liquors obtained after isolation of jaceosidin were combined and the resulting residue (6 g) was chromatographed on silica gel (180 g) developed with a C_6H_6–EtOAc gradient system. Fractions 26–34, eluted with C_6H_6–EtOAc (7:3), yielded, after treatment with EtOAc–Et_2O, a product which was recrystallized from Me_2CO–*iso*-Pr_2O to give 100 mg **6**, mp 230°. An additional 53 mg **6** were obtained from CC on silica gel of the mother liquors. The total yield of **6** was 153 mg (0.0031 % of the dry wt). IR ν_{max}^{KBr} cm^{-1}: 3500, 3425, 1755, 1135, 1110, 1000, 940; UV λ_{max}^{MeOH} nm (ε): 207 (8959); $[\alpha]_D = -119$ 5; (found: C, 57 02; H, 6 22; Cl, 11 45 %. $C_{15}H_{19}ClO_5$ requires: C, 57.32; H, 6.05; Cl, 11.14 %); CIMS *m/z* (rel int). 317 [M + 3]$^+$ (30), 315 [M + 1]$^+$ (100), 299 (15), 297 [M + 1 − H_2O]$^+$ (45.2), 281 (10), 279 [297 − H_2O]$^+$ (31), 261 [297 − HCl]$^+$ (25), 243 [279 − HCl]$^+$ (17).

Isolation of desacetyl matricarin (**2**) 2 g of **2** crystallized spontaneously from fractions 113–123 of the initial column. Percolation over tonsil (20 g) of the mother liquors using as eluents C_6H_6, C_6H_6–EtOAc and EtOAc, gave an additional 1 g of **2** Finally, further CC of the second mother liquors yielded a further 200 g of **2**. The total yield of **2** was 3 2 g (0 0649 % of dry wt), mp 151°. The solid was identified as such by standard procedures

Isolation of chrysartemin A (**3**) Fractions 124–150 (5.2 g), eluted with C_6H_6–EtOAc (13:7), were rechromatographed on silica gel (200 g) using as eluent EtOAc–C_6H_6 in different proportions. From fractions 101–107, eluted with EtOAc–C_6H_6 (1.1), crystallized 75 mg (0.0021 % of dry wt) of **3**, mp 245° Compound **3** was identical with an authentic specimen.

Isolation of ridentin (**5**). Fractions 151–175, eluted with C_6H_6–EtOAc (1:1) (10 g), were chromatographed on tonsil (180 g) and eluted with C_6H_6–Me_2CO in increasingly polar ratios. Fraction 22, eluted with Me_2CO, gave 200 mg **5**, mp 205° (lit. mp 215–218° [7], 208–211° [9]) An additional 150 mg were obtained from fractions 176–207 from the original column. These fractions were rechromatographed on a column packed with silica gel (310 g). On elution with EtOAc, fractions 24–32 gave pure lactone **5**. The total yield was 350 mg (0 0071 % of the dry wt). IR ν_{max}^{KBr} cm^{-1}: 3340, 1765, 1305, 1155, 988, 975, 960, EIMS *m/z* (rel int.): 264 [M$^+$, $C_{15}H_{20}O_4$] (20), 91 (89.7), 53 (100), 43 (82), 41 (88).

Acetylation of **5**. Compound **5** (100 mg) was acetylated with Ac_2O (1 ml) in C_5H_5N (1 ml). The reaction mixture was monitored as usual. The acetylated product was obtained as a glassy solid and it was used for decoupling experiments ^{1}H NMR ($CDCl_3$, 80 MHz)· δ1.71 (*d*, 3H, $J = 1$ 8 Hz, H-15), 2.00 (*s*, 3H, H-16), 2 07 (*s*, 3H, H-17), 4.37 (*t*, 1H, $J = 11$ Hz, H-6), 5.03 (*s* (*br*), 2H, H-14), 5 27 (*c*, 1H, $J = 1.8$ Hz, H-5), 5 45 (*d*, 1H, $J = 3$ Hz, H-13a), 6.17 (*d*, 1H, $J = 3$ Hz, H-13b)

Acetylation of jaceosidin (**8**) Compound **8** (108 mg) was acetylated with Ac_2O–C_5H_5N. The acetylated product was crystallized from Me_2CO as colourless needles (yield 90 mg), mp 225° (lit mp 220° [16]); IR ν_{max}^{KBr} cm^{-1} 1764, 1643, 1617, 1591, 1478, 1421, 1266, 1073. EM *m/z* (rel int.): 414 [M]$^+$ (61.4), 352 (30), 330 (48.4), 312 (66.3), 43 (100)

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REFERENCES

1. Kelsey, R. G. and Shafizadeh, F. (1979) *Phytochemistry* **18**, 1591
2. Romo, J., Romo de Vivar, A., Treviño, R., Joseph-Nathan, P. and Díaz, E. (1970) *Phytochemistry* **9**, 1615.
3. Romo de Vivar, A., Vázquez, F. and Zetina, C. (1977) *Rev. Latinoam. Quím.* **8**, 127.
4. Mata, R., Delgado, G. and Romo de Vivar, A. (1984) *Phytochemistry* **23**, 1665.
5. Zarikov, S. K., Kasymov, S. Z., Rakhamankulov, U. and Sidyakin, G. P. (1976) *Khim. Prir Soedin.* 397.
6. Tori, K., Horibe, I., Tamura, Y and Toda, H. (1973) *J. Chem. Soc Chem. Commun.* 620.
7. Irwin, M. A., Lee, K. H., Simpson, R. F. and Geissman, T. A. (1969) *Phytochemistry* **8**, 2009.
8. Irwin, M. A. and Geissman, T A. (1973) *Phytochemistry* **12**, 871.
9. Liu, Y. L and Mabry, T. J. (1981) *J Nat. Prod.* **44**, 722.
10. Lee, K. H., Simpson, R F. and Geissman, T A. (1969) *Phytochemistry* **8**, 1515.
11. Bhadane, N. R and Shafizadeh, F (1975) *Phytochemistry* **14**, 2651
12. Tori, K., Kitahoki, K., Takano, Y, Tanida, H. and Tsuji, T. (1964) *Tetrahedron Letters* 559.
13. Samek, Z. and Budesinsky, M. (1979) *Collect. Czech. Chem. Commun.* **44**, 558.
14. Grover, S. H., Guthrie, J. P., Stothers, J. B. and Tan, C. T. (1973) *J. Magn. Reson.* **10**, 227.
15. Irwin, M. A. and Geissman, T. A. (1973) *Phytochemistry* **12**, 863.
16. ApSimon, J. W., Haynes, N. B., Sim, K. Y. and Whalley, W. B. (1963) *J. Chem. Soc.* 3780.
17. Wagner, H., Horhamer, L., Hoer, R., Murakani, T and Farkas, L. (1969) *Tetrahedron Letters* 3411.
18. Liu, Y. L. and Mabry, T. J. (1981) *Phytochemistry* **20**, 1389
19. Hori, T., Masamura, M. and Okomura, F. S. (1961) *Bull. Chem. Soc. Japan* **34**, 1547.
20. Farkas, L., Nogradi, M. and Zubovics, Z. (1967) *Acta Chim. Acad. Sci. Hung.* **52**, 301.
21. Farkas, L., Nogradi, M, Sudarsanam, V. and Herz, W. (1967) *Tetrahedron* **23**, 3557.
22. Osawa, T., Suzuki, A. and Tamura, S. (1974) *Tetrahedron Letters* 5135.
23. González, A. G., Bermejo, J., Bretón, J. L., Massanet, G. M. and Triana, J. (1974) *Phytochemistry* **13**, 1193
24. Kupchan, S M., Kelsey, J. E., Maruyama, M., Cassady, J. M., Hemingway, H. and Knox, J. R. (1969) *J. Org. Chem.* **34**, 3876.
25. Silva, A. R., García, M., Baker, P. M. and Rabi, J A. (1981) *Org. Magn. Reson.* **16**, 230.