Pergamon

PII: S0031-9422(96)00517-1

Phytochemistry, Vol. 44, No. 3, pp. 479–483, 1997

0031-9422/97 $17.00 + 0.00

DITERPENES FROM *HYALIS ARGENTEA*

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(*Received in revised form* 27 *June* 1996)

Key Word Index—*Hyalis argentea*; Mutisieae; Compositae; ent-kaurene; diterpene glycosides; sequiterpene lactones; guaianolides.

Abstract—The aerial parts of *Hyalis argentea* yielded, in addition to four known ent-kaurenes, three known guaianolides and one coumarin, the new (7*R*)-ent-15-oxokaur-16-en-7-ol-19-oic acid β-glucopyranosyl ester and three new diterpene lactones which biosynthetically seem to be derived from ent-15-oxokaurenoic acid. The structures were determined mainly by one- and two-dimensional spectroscopy. Copyright © 1997 Elsevier Science Ltd

INTRODUCTION

The genus *Hyalis* [1] is distributed in the South American continent from central and northern Argentina to some regions of Bolivia and Paraguay [2]. To our knowledge, there is no previous report on the chemical constituents of any member of this small genus [3], a situation which made it attractive to carry out the present work. This paper describes the isolation and structural elucidation of the constituents of *Hyalis argentea* [2], a shrub which grows in the dunes of north-western Argentina close to the Andean cordillera from Mendoza to Jujuy. The aerial parts of this plant contain four new substances: the ent-kaurene glycoside **1** and the three diterpene lactones, **2**–**4**. In addition, the known ent-kaurenes **5** and **6** [4], the ent-kaurene glycosides paniculoside II (**7**) and paniculoside III (**8**) [5], zaluzanin C [6], glucozaluzanin C [7], 11,13-dihydro-3-glucozaluzanin C [8], and 7-hydroxycoumarin [9] were isolated, thus providing a significant string of secondary metabolites which are useful in understanding the biogenetic pathway in this species.

RESULTS AND DISCUSSION

The ^{1}H and ^{13}C NMR spectra of compound **1** revealed that this substance differs from compound **8** [5] only in the position of the hydroxyl group. Location of this group at C-7 was deduced from the ^{1}H–^{1}H COSY contour plot, where the fragment CH(5)-CH_2(6)-CHOH(7) could be clearly recognized. In addition, the ^{13}C NMR signal for the quaternary carbon C-8 appeared at δ 59.0, which is *ca* 6 ppm downfield from the shift of the same carbon in ent-15-oxokaur-16-en-19-oic acid (**9**) isolated from *Pteris longipes* [10], due to the presence of the adjacent hydroxyl group. Inspection of the molecular model depicted in Fig. 1(a) in combination with the coupling constants $J_{6ax,7ax}$ = 12.7 and $J_{6ec,7ax}$ = 4.1 Hz, allowed the stereochemistry of the hydroxyl group at C-7 to be determined as shown in **1**. The presence of a glucose moiety was evident from the ^{13}C chemical shifts, when compared with data for other glucopyranosyl esters [5].

Most of the ^{13}C chemical shifts of compound **2** (Table 1) closely resemble those of compound **9** [10]. However, the presence of a quaternary carbon signal at δ 85.0 (C-8), which is found at δ 52.6 in compound **9** and the presence of a carbonyl group signal of an α,β-unsaturated lactone at δ 166.3 instead of δ 209.6 for the α,β-unsaturated ketone of compound **9**, clearly indicated that an oxygen atom was located between C-8 and C-15. In addition, the signals for the vinylic carbons C-16 and C-17 are shifted from δ 150.2 and 113.8 in compound **9** to δ 139.4 and 126.9 in compound 2, as expected for the double bond carbons of an α,β-unsaturated lactone. From the chemical point of view, lactone **2** corresponds to the Baeyer–Villiger oxidation product of compound **9**. This transformation breaks a carbon–carbon bond of the ent-kaurane framework, giving a substance whose hydrolysis leads to a new skeleton. As far as we know, this is the first report on such functionalization in nature and, therefore, compound **2** is named hyalic acid.

The structure of compound **3** followed from its ^{1}H and ^{13}C NMR data, which indicated the presence of a

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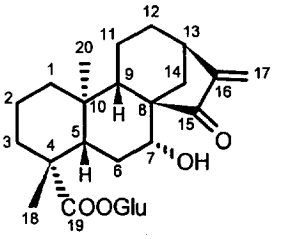
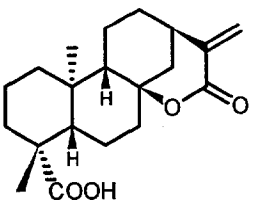
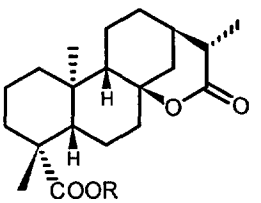
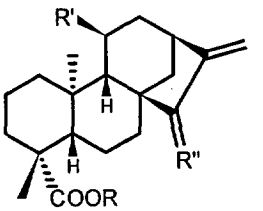


1



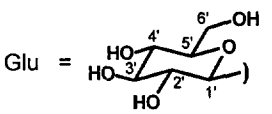
2



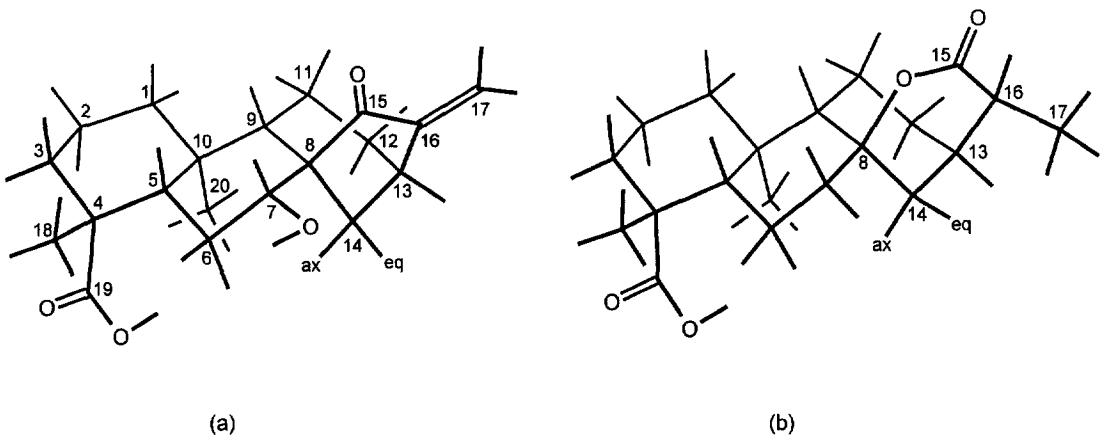


3 : R = H
4 : R = Glu

5 : R = H; R' = OH; R" = (⁻OH; ····H)
6 : R = H; R' = OH; R" = O
7 : R = Glu; R' = OH; R" = (⁻OH; ····H)
8 : R = Glu; R' = OH; R" = O
9 : R = H; R' = H; R" = O



$CH–CH_3$ moiety instead of the exocyclic double bond present in compound **2**. Therefore, compound **3** is dihydrohyalic acid. In order to determine the stereochemistry at C-16, we obtained the minimum energy conformations of structure **3** (Fig. 1(b)) and that of its epimer at C-16 by means of MMX calculations [11]. The calculated dihedral angle H-C(13)-C(16)-H for compound **3** was 84°, while for its C-16 epimer it was



(a) (b)

Fig 1. MMX molecular models of (7*R*)-ent-15-oxokaur-16-en-7-ol-19-oic acid (a) and (16*S*)-dihydrohyalic acid (**3**) in the minimum energy conformation.

Table 1. ^{13}C NMR spectral data of compounds **1–4** (75.4 MHz, TMS as internal standard)

C	1*	2†	3†	4*
1	40.3	41.1	41.0	41.1
2	19.3	19.2	19.1	19.6
3	38.2	37.5	37.4	38.0
4	44.0	43.6	43.6	44.0
5	54.0	56.2	56.2	56.6
6	30.8	20.7	20.7	21.1
7	71.1	33.9	29.7‡	29.6‡
8	58.9	85.0	84.7	84.5
9	51.8	51.3	51.6	52.2
10	40.0	40.0	39.9	40.2
11	18.6	16.5	16.6	16.9
12	33.1	30.6	29.2‡	29.2‡
13	38.2	34.3	33.3	33.6
14	28.8	42.4	42.3	42.9
15	209.6	166.3	176.4	175.5
16	150.2	139.4	40.7	41.2
17	113.4	126.9	19.9	19.9
18	28.4	29.0	29.0	28.5
19	176.7	182.2	183.2	176.7
20	15.9	16.5	16.3	16.4
1′	95.8	–	–	95.9
2′	73.9	–	–	74.2
3′	79.2	–	–	79.1
4′	71.1	–	–	71.1
5′	79.4	–	–	79.4
6′	62.2	–	–	62.2

*In pyridine-d_5.
†In $CDCl_3$.
‡May be interchanged.

46°. These angles allowed us to estimate for each case the expected coupling constant by using a generalized Karplus-type equation [12, 13], which gave 0.5 Hz for compound **3** and 5.5 Hz for the C-16 epimer. Because the signal for H-16 appeared as a slightly broadened quartet due to coupling with Me-17 (7 Hz) and a long-range coupling with H-14ax (1 Hz), which were confirmed by irradiation of H-16 and H-14ax, the experimental value of $J_{13,16}$ must be almost zero, and therefore, the stereochemistry at C-16 is as in compound **3**.

The structure of glycoside **4** was readily assigned when its ^1H NMR spectrum was compared with that of compound **3**. In addition, the ^{13}CNMR data confirmed the presence of the glucose moiety.

The ^1H and ^{13}C NMR assignments of compounds **1–4**, listed in the Experimental and in Table 1, are supported by the information provided by COSY contour plots determined for the four compounds, by DEPT spectra of compounds **1–3**, by an APT of compound **4** and by a heteronuclear chemical shift correlation diagram of compound **1**. The identity of the known substances was determined by comparison of their ^1H or ^{13}C NMR chemical shifts with literature values [4–8].

The optical rotations of the known ent-kaurene derivatives isolated in this work (**5–8**) are identical to those found in *Eupatorium album* [4] and *Stevia paniculata* [5], which are ent-derivatives. Therefore, based on biogenetic considerations, we assume that the new substances **1–4** also possess the same absolute stereochemistry. From the biogenetic point of view, it is worth mentioning that bridged δ-lactone functional groups, such as those in compounds **2–4**, are rare in nature. They were found in the *Zaluzania augusta* sesquiterpene lactones zaluzanins A and B [14] that occur together with zaluzanin C [6] found in this work. Finally, from the potential therapeutic point of view it is relevant that compound **9** and a deglucose C-7 epimer of compound **1** show HIV-inhibitory activity toward HIV-1 infected cells [15–17].

EXPERIMENTAL

General. HPLC with a differential refractometer was used for sepns. The columns employed were: (A) Phenomenex Ultremex C18 (5μ, 10 × 250 mm) and (B) Beckman Ultrasphere C18 (5μ, 10 × 250 mm). R_t measured from the solvent peak.

Plant material. Aerial parts of *H. argentea* Don ex Hook. et Arn. var. *argentea* were collected at the flowering stage in January 1994 near the town of Santa María, Catamarca province, Argentina. A voucher specimen (LILL No. 597325) is deposited at Herbario de la Fundación Miguel Lillo, Tucumán, Argentina.

Extraction and isolation. Leaves and heads were processed separately. Heads (200 g) were extracted (× 2) with $CHCl_3$ (1.3 l) at room temp. for 4 days to give 1.6 g of crude extract after evapn under vacuum. CC over silica gel (90 g) using hexane with increasing amounts of EtOAc (0–50%) yielded 60 frs which were monitored by TLC. Frs 38–42 (50.2 mg) were processed by HPLC (column A, MeOH, 2 ml min^{-1}) to give 10.9 mg of lupeol. Frs 50–60 (89.6 mg) were processed by HPLC (column A, MeOH, 2 ml min^{-1}) to give 4 mg of stigmasterol (R_t 41.5 min) and 5.8 mg of sitosterol (R_t 46 min).

Leaves (500 g) were extracted (× 2) with MeOH (2 l) at room temp. for 4 days to give 117 g of crude extract which was suspended in MeOH (1.2 l) at 50°, diluted with H_2O (1.2 l) and extracted (× 2) with hexane (900 ml) and (× 2) with $CHCl_3$ (900 ml). Evapn of the $CHCl_3$ extracts furnished an oily residue (21 g). CC over silica gel (525 g) of a portion of this residue (12 g) using $CHCl_3$ with increasing amounts of EtOAc (0–50%) followed by $CHCl_3$–EtOAc–MeOH (10:10:3) yielded 90 frs which were monitored by TLC. Frs 11–15 (108 mg) were processed by HPLC (column A, MeOH–H_2O 2:1, 1.7 ml min^{-1}) to give 6.5 mg of 7-hydroxycoumarin [9] (R_t 3 min), 43 mg of compound **3** (R_t 21.3 min) and 7.6 mg of compound **2** (R_t 24.5 min). Frs 16–25 were combined (174 mg) and processed by HPLC (column A, MeOH–H_2O, 2:1 1.7 ml min^{-1}) to yield 15 mg of compound **5** (R_t 13.5 min), and enriched fractions of compounds **3** and **6**, which were purified by rechromatography. Compound **3** (2.4 mg) on column A (MeOH–H_2O, 3:2, 2 ml min^{-1}, R_t 48.7 min) and compound **6** (3.5 mg) on

column B (MeOH–H_2O, 4:3, 2 ml min^{-1}, R_t16.7 min). Frs 27–34 (76.9 mg) were processed by HPLC (column A, MeOH–H_2O, 2:1, 2 ml min^{-1}) to give 8.8 mg of zaluzanin C [6] (R_t 6 min). A portion (550 mg) of frs 86–90 (2.7 g) was processed by HPLC (column B, MeOH–H_2O, 3:2, 2 ml min^{-1}) to yield 30 mg of glucozaluzanin C [7] (R_t 10.5 min), 20.3 mg of compound **1** (R_t 47 min), and four more components which were purified by rechromatography (column B, MeOH–H_2O, 1:1, 2 ml min^{-1}) to give 30 mg of compound **8** (R_t 6.5mg), 21.3 mg of compound **7** (R_t 13.5 min), 6.1 mg of 11,13-dihydro-3-glucozaluzanin C [8] (R_t 22.5 min) and 3.7 mg of **4** (R_t 58 min), respectively.

(7R)-*ent*-15-*Oxokaur*-16-*en*-7-*ol*-19-*oic acid β-glucopyranosyl ester* (**1**). Solid, mp 163–165°; UV λ_{max}^{EtOH} nm (log ε): 238 (3.83); IR ν_{max}^{KBr} cm^{-1}: 3400, 3050, 1730, 1650, 1225, 1055; $[\alpha]_{589}$ −106° (MeOH; *c* 2.6); ^1H NMR (300 MHz, pyridine-d_5); δ 6.28 (1H, *d*, $J_{1',2'}$ = 8 Hz, H-1′), 6.04 (1H, *br s*, H-17), 5.16 (1H, *br s*, H-17′), 4.45 (1H, *dd* $J_{6ax,7ax}$ = 13, $J_{6eq,7ax}$ = 4Hz, H-7), 4.43-4.30 (2H, 2 *m*, H-6a′ and H-6b′), 4.30 (1H, *t*, $J_{2',3'} \approx J_{3',4'} \approx$ 9 Hz, H-3′), 4.25 (1H, *t*, $J_{3',4'} \approx J_{4',5'} \approx$ 9 Hz, H-4′), 4.19 (1H, *br t*, $J_{1',2'} \approx J_{2',3'} \approx$ 8 Hz, H-2′), 4.02 (1H, *ddd*, $J_{4',5'}$ = 9, $J_{5',6a'}$ = 4, $J_{5',6b'}$ = 3, H-5′), 2.91 (1H, *m*, H-13), 2.76 (1H, *q*, $J_{5,6ax} \approx J_{6ax,6eq} \approx J_{6ax,7ax} \approx$ 13 Hz, H-6ax) 2.53 (1H, *ddd*, $J_{5,6eq}$ = 2, $J_{6ax,6eq}$ = 13, $J_{6eq,7}$ = 4 Hz, H-6eq), 2.49 (1H, *ddd*, $J_{12eq,14eq}$ = 1, $J_{13,14eq}$ = 5 Hz, $J_{14ax,14eq}$ = 12 Hz, H-14eq), 2.37 (1H, *br d*, $J_{3ax,3eq}$ = 13 Hz, H-3eq), 2.32 (1H, *d*, $J_{14ax,14eq}$ = 12 Hz, H-14ax), 2.19 (1H, *qt*, $J_{1ax,2ax} \approx J_{2ax,2eq} \approx J_{2ax,3ax} \approx$ 14, $J_{1eq,2ax} \approx J_{2ax,3eq} \approx$ 3 Hz, H-2ax), 1.79 (1H, *m*, H-11), 1.74 (1H, *br d*, $J_{1ax,1eq}$ = 14 Hz, H-1eq), 1.62-1.42 (3H, *complex m*, H-11′, H-12 and H-12′), 1.42 (1H, *m*, H-2eq), 1.36 (1H, *d*, $J_{9,11ax}$ = 8 Hz, H-9), 1.35 (3H, *s*, Me-20), 1.30 (1H, *dd*, $J_{5,6ax}$ = 12, $J_{5,6eq}$ = 1 Hz, H-5), 1.25 (3H, *s*, Me-18), 1.01 (1H, *td*, $J_{2ax,3ax} \approx J_{3ax,3eq} \approx$ 13, $J_{2eq,3ax}$ = 4 Hz, H-3ax), 0.69 (1H, *td*, $J_{1ax,1eq} \approx J_{1ax,2ax} \approx$ 14, $J_{1ax,2eq}$ = 4 Hz, H-1ax).

Hyalic acid (**2**). Solid, mp 160–163°; UV λ_{max}^{EtOH} nm (log ε): 214 (3.77); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3512, 3180, 1704, 1604, 1218; $[\alpha]_{589}$ −79°, $[\alpha]_{578}$ −82°, $[\alpha]_{546}$ −95°, $[\alpha]_{436}$ −158°, $[\alpha]_{365}$ −245° ($CHCl_3$; *c* 0.38); ^1H NMR (300 MHz, $CDCl_3$): δ 6.43 (1H, *d*, $J_{17,17'}$ = 2 Hz, H-17), 5.54 (1H, *br s*, H-17′), 2.93 (1H, *m*, H-13), 2.32 (1H, *dd*, $J_{13,14ax}$ = 3, $J_{14ax,14eq}$ = 14 Hz, H-14ax), 2.21 (1H, *br d*, $J_{3ax,3eq}$ = 14 Hz, H-3eq), 1.28 (3H, *s*, Me-18), 1.06 (1H, *td*, $J_{2ax,3ax} \approx J_{3ax,3eq} \approx$ 13, $J_{2eq,3ax}$ = 4 Hz H-3ax), 0.98 (1H, *m*, H-lax), 0.96 (3H, *s*, Me-20); EI-MS (20 eV) *m/z* (rel. int): 332 $[M]^+$ (10), 286 (69), 275 (37), 227 (26), 167 (52), 164 (55), 121 (100).

(16S)-*Dihydrohyalic acid* (**3**). Solid, mp 198–199°; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3508, 3074, 1700, 1266, 1218; $[\alpha]_{589}$ −103°, $[\alpha]_{578}$ −107°, $[\alpha]_{546}$ −121°, $[\alpha]_{436}$ −200°, $[\alpha]_{365}$ −307° ($CHCl_3$; *c* 0.77); ^1H NMR (300 MHz, $CDCl_3$): δ 2.49 (1H, *br q*, $J_{16,17}$ = 7 Hz, H-16), 2.20 (1H, *br d*, $J_{3ax,3eq}$ = 14 Hz, H-3eq), 2.16 (1H, *ddd*, $J_{13,14ax,}$ = 4, $J_{14ax,14eq}$ = 14, $J_{14ax,16}$ = 1 Hz H-14ax), 1.94 (1H, *br m*, H-13), 1.91 (1H, *br d*, $J_{1ax,1eq}$ = 14 Hz, H-1eq), 1.84 (1H, *complex m*, H-2ax), 1.71 (1H, *br d*, $J_{14ax,14eq}$ = 14 Hz, H-14eq), 1.48 (1H, *complex m*, H-2eq), 1.35 (3H, *d*, $J_{16,17}$ = 7 Hz, Me-17), 1.28 (3H, *s*, Me-18), 1.27 (1H, *d*, $J_{9,11ax}$ = 8 Hz, H-9), 1.19 (1H, *dd*, $J_{5,6ax}$ = 12, $J_{5,6eq}$ = 1 Hz, H-5) 1.04 (1H, *td*, $J_{2ax,3ax} \approx J_{3ax,3eq} \approx$ 13, $J_{2eq,3ax}$ = 4 Hz H-3ax), 0.95 (3H, *s*, Me-20), 0.93 (1H, *m*, H-1 ax) EI-MS (20 eV) *m/z* (rel. int): 290 $[M - COO]^+$ (36), 275 (100), 229 (33), 173 (13), 135 (18), 121 (28).

(16S)-*Dihydrohyalic acid β-glucopyranosyl ester* (**4**). Gum; IR ν_{max}^{film} cm^{-1}: 3368, 2928, 2870, 1736, 1710, 1268, 1230, 1070; $[\alpha]_{589}$ −60° (MeOH; *c* 0.4); ^1H NMR (300 MHz, pyridine-d_5): δ 6.22 (1H, *d*, $J_{1',2'}$ = 8 Hz, H-1), 4.48 (1H, *br dd*, $J_{5',6a'}$ = 3 Hz, $J_{6a',6b'}$ = 12 Hz, H-6a′), 4.40 (1H, *br m*, H-6b′), 4.37 (1H, *br t*, $J_{2',3'} \approx J_{3',4'}$ 9 Hz, H-3′), 4.26 (1H, *t*, $J_{3',4'} = J_{4',5'}$ = 9 Hz, H-4′), 4.21 (1H, *br t*, $J_{1',2'} \approx J_{2',3'} \approx$ 8 Hz, H-2′), 4.04 (1H, *ddd*, $J_{4',5'}$ = 9, $J_{5',6b'}$ = 4, $J_{5'6a'}$ = 3 Hz, H-5′), 2.49 (1H, *br q*, $J_{16,17}$ = 8 Hz, H-16), 2.37 (1H, *br d*, $J_{3ax,3eq}$ = 13 Hz, H-3eq), 2.34 (1H, *dq*, $J_{5,6ax} \approx J_{6ax,6eq} \approx J_{6ax,7ax} \approx$ 13, $J_{6ax,7eq}$ = 4Hz, H-6ax), 2.15 (1H, *br d*, $J_{14ax,14eq}$ = 14 Hz, H-14ax), 2.06 (1H, *br m*, H-2ax), 2.03 (1H, *br d*, $J_{6ax,6eq}$ = 14 Hz, H-6eq), 1.92 (1H, *dt*, $J_{6ax,7eq} \approx J_{6eq,7eq} \approx$ 3 Hz, $J_{7ax,7eq}$ = 13, H-7eq), 1.76 (1H, *td*, $J_{7ax,6ax} \approx J_{7ax,7eq} \approx$ 13, $J_{7ax,6eq}$ = 4 Hz, H-7ax), 1.74 (1H, *br d*, $J_{1ax,1eq}$ = 14 Hz, H-1eq), 1.63 (1H, *br m* H-13), 1.59 (1H, *complex m*, H-11ax), 1.44 (1H, *m*, H-2eq), 1.43 (1H, *br d*, $J_{14ax,14eq}$ = 14 Hz, H-14eq), 1.31 (1H, *d*, $J_{9,11ax}$ = 8 Hz, H-9), 1.26 (3H, *s*, Me-18), 1.25 (3H, *d*, $J_{16,17}$ = 8 Hz, Me-17), 1.17 (1H, *br d*, $J_{5,6ax}$ = 12 Hz, H-5) 1.15 (3H, *s*, Me-20), 1.00 (1H, *td*, $J_{2ax,3ax} \approx J_{3ax,3eq} \approx$ 13, $J_{2eq,3ax}$ = 4 Hz, H-3ax), 0.82 (1H, *td*, $J_{1ax,1eq} \approx J_{1ax,2ax} \approx$ 14, $J_{1ax,2eq}$ = 4 Hz, H-1 ax).

Acknowledgements—The work in Tucumán was supported by grants from Consejo Nacional de Investigaciones Científicas y Técnicas (Argentina) and Consejo de Investigaciones de la Universidad Nacional de Tucumán (CIUNT). Partial financial support from CoNaCyT, México, and stimulating support from CYTED, Spain, is also acknowledged.

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