1304, 1334, 1374, 1428, 1464, 1572, 1610, 1640, 1708, 2990, 3400 ^{1}H NMR (100 MHz, $CDCl_3$) δ0 80 (*t*, 3H, CH_2–Me), 1 20 (*m*, 6H, $(CH_2)_3$–Me), 1 50 (*s*, 9H, CMe_3), 2 34 (*t*, 2H, CO–CH_2–CH_2) 3 72 (*s*, 3H, OMe), 3 93 (*s*, 2H, benzyl CH_2), 6 13, 6 32 (2 × *d*, 2H, 3-H, 5-H), 11 64 (*s*, 1H, OH)

Tert-*butyl everninate* (**19**) Crystals, mp 28° (from *n*-pentane) $C_{13}H_{18}O_4$ (238 3) IR ν_{max}^{KBr} cm^{-1} 700, 758, 818, 850, 952, 992, 1040, 1062, 1118, 1160, 1200, 1262, 1300, 1330, 1370, 1420, 1450, 1576, 1610, 1640, 3000, 3450 ^{1}H NMR (200 MHz, $CDCl_3$) δ2 80 (*s*, 9H, CMe_3), 3 45 (*s*, 3H, Me), 4 45 (*s*, 3H, OMe), 6 39, 6 45 (2 × *d*, 2H, 3-H, 5-H), 10 80 (*s*, 1H, OH)

Tert-*butyl β-orcinolcarboxylate* (**21**) Prisms, mp 128–130° (from Et_2O–*n*-hexane) $C_{14}H_{18}O_4$ (250 3) IR ν_{max}^{KBr} cm^{-1} 730, 842, 966, 1024, 1058, 1100, 1140, 1158, 1248, 1300, 1368, 1394, 1430, 1450, 1590, 1620, 1640, 3000, 3480 ^{1}H NMR (100 MHz, $CDCl_3$) 1 55 (*s*, 9H, CMe_3), 2 04 (*s*, 3H, 3-Me), 2 36 (*s*, 3H, 6-Me), 5 50 (*br s*, 1H, 4-OH), 12 18 (*s*, 1H, 2-OH)

Tert-*butyl* 4-O-*methyloliveto1carboxylate* (**22**) Oil $C_{17}H_{26}O_4$ (294 4) IR ν_{max}^{film} cm^{-1} 710, 754, 780, 820, 832, 850, 960, 1042, 1110, 1154, 1194, 1260, 1300, 1330, 1370, 1422, 1462, 1570, 1606, 1636, 2970, 3400 ^{1}H NMR (100 MHz, $CDCl_3$) δ0 83 (*t*, 3H, CH_2–Me), 1 28 (*m*, $(CH_2)_3$–Me), 1 56 (*s*, 9H, CMe_3), 2 80 (*t*, 2H, benzyl CH_2), 6 17, 6 23 (2 × *d*, 2H, 3-H, 5-H), 11 84 (*s*, 1H, OH)

REFERENCES

1 Bachelor, F W, Cheriyan, U O and Wong, J D (1979) *Phytochemistry* **18**, 487
2 Meyyappan, A, Neelakantan, S and Ramesh, P (1981) *Curr Sci* **50**, 1028
3 Huneck, S (1984) *Phytochemistry* **23**, 431

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FLAVONOIDS FROM *ACHYROCLINE FLACCIDA*

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Key Word Index—*Achyrocline flaccida*, Compositae, Inuleae, aerial parts, prenylated flavonoids, flavonoids, caffeic acid derivatives

Abstract—Three new flavonoids 5-hydroxy-7-(3-methyl-2,3-epoxybutoxy)flavanone, 5-hydroxy-3,8-dimethoxy 7-(3-methyl-2,3-epoxybutoxy)flavone and 4′-hydroxy-5-methoxy-7-(3-methyl-2,3-epoxybutoxy)flavone were isolated and identified from the aerial parts of *Achyrocline flaccida* Tamarixetin, gnaphalin, isognaphalin, 5,7,8-trihydroxy-3-methoxyflavone, chrysoeriol, galangin 3-methyl ether, naringenin 5-methyl ether, caffeic acid, chlorogenic acid and isochlorogenic acid were also isolated

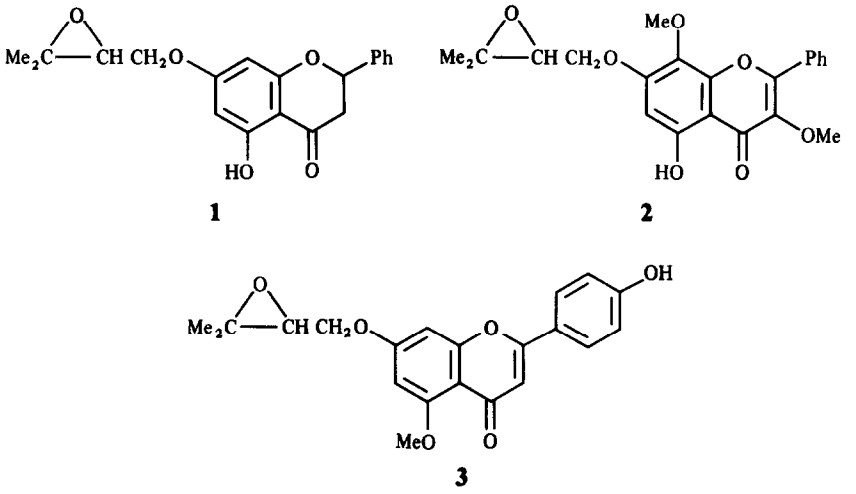
INTRODUCTION

In continuation of our chemosystematic search of the tribe Inuleae (Compositae), we have now investigated *Achyrocline flaccida* (Weinm) DC, a shrub, widely distributed in the North of Argentina and the South of Brazil In a previous paper we reported the identification of galangin, galangin 3-methyl ether, quercetin 3-methyl ether and two esters of calleryanin (3,4-dihydroxybenzyl alcohol 4-glucoside) with caffeic acid and protocatechuic acid from *Achryrocline satureioides* [4] Investigation of the acetone extract of *A flaccida* resulted in the isolation and determination of the structure of 7,4′-dihydroxy-5-methoxyflavanone and the corresponding 4,2′4′-trihydroxy-6-methoxychalcone [5]

The most characteristic features distinguishing members of the Inuleae from those of other Compositae tribes is the presence of flavonols lacking B ring hydroxylation, 6 and/or 8 hydroxyflavonols and their methyl ethers [6] In the present report we describe the occurrence of such typical flavonoids, together with the identification of three new prenylated flavonoids

RESULTS AND DISCUSSION

The hexane extract of the aerial parts of *A flaccida* was subjected to silica gel CC affording three new flavonoids The first of these, compound 1 showed a brown colour in UV (365 nm) and a yellow–green colour with methanolic ferric chloride Its UV spectrum exhibited maxima at 272 and 280 (sh) nm characteristic of a flavanone The shifts induced in the UV spectra by aluminium chloride, sodium acetate and sodium methoxide led us to conclude that there is only one free hydroxyl attached to C-5 The ^{1}H NMR spectrum (in $CDCl_3$) showed a multiplet at δ7 6 characteristic of an unsubstituted aromatic ring (B ring), δ6 2 and 5 8 signals from protons H-6 and H-8 δ5 3 corresponding to H-2 and δ2 6 (multiplet) to H-3 *trans* and H-3 *cis* The aliphatic chain showed the gem-dimethyl



1

2

3

signal at 1 1 and at 4 45 the OCH_2 signal IR spectrum suggested the presence of an epoxy group The molecular ion in the mass spectrum at $C_{20}H_{20}O_5$ ($[M]^+$ 340) and mass spectral fragments at m/z 255 $[M-85]^+$ confirmed its structure as **1**

The second new flavonoid (**2**) showed a brown colour in UV and a yellow–green colour with methanolic ferric chloride Its UV spectrum exhibited maxima at 271, 285 sh and 350 nm The bathochromic shifts with aluminium chloride, sodium acetate and sodium methoxide led us to conclude that there is only one hydroxyl attached to C-5 and its structure is similar to 7-methylgnaphalin The ^1H NMR spectrum (in $CDCl_3$) showed a multiplet at δ7 6 characteristic of an unsubstituted aromatic ring (B ring), two methoxyl groups at δ4 4 assigned to C-3 and C-8 and at δ6 35 the H-6 The aliphatic chain showed the gem-dimethyl signal at δ4 45 the OCH_2 signal IR spectrum suggested the presence of an epoxy group The molecular ion in the mass spectrum at $C_{22}H_{22}O_7$ ($[M]^+$ 378) and mass spectral fragments at m/z 293 $[M-85]^+$ confirmed this structural assignment

The third flavonoid (**3**) showed a blue colour in UV and a yellow colour with methanolic ferric chloride Its UV spectrum exhibited maxima at 281 and 329 nm The shifts induced by aluminium chloride, sodium acetate and sodium methoxide led us to conclude that **3** is a flavone with a single hydroxyl group attached to C-4′ ^1H NMR spectrum (in $CDCl_3$) showed a typical four-peak pattern of two doublets for ring B oxygenated at C-4′, at δ6 2 and 5 2 signals from protons at C-6 and C-8, at δ6 4 the singlet corresponding to proton at C-3 The aliphatic chain showed the gem-dimethyl signal at 1 1 and at 4 45 the OCH_2 signal The IR spectrum suggested the presence of an epoxy group The molecular ion in the mass spectrum at $C_{21}H_{20}O_6$ ($[M]^+$ 368) and mass spectral fragments at m/z 283 $[M-85]^+$ confirmed the structure

EXPERIMENTAL

Mps are uncorr ^1H NMR were recorded at 80 MHz in $CDCl_3$ and DMSO-d_6 (TMS as int standard) CC was carried out with silica gel (Merck) and Polyclar Analytical TLC was performed on silica gel G (Merck)

Material Achyrocline flaccida (Weinm) DC (Compositae) was collected in Colonia Benítez, Chaco Province and identified by Dr A Schultz (INTA) Voucher specimen is deposited in the University Herbarium (Museo de Botánica, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires)

Extraction. Air dried ground aerial parts of *A flaccida* (1 6 kg) were extracted successively with hexane, C_6H_6, CH_2Cl_2, Me_2CO and MeOH in a Soxhlet The hexane extract was passed through a Sephadex LH 20 column for purification, the C_6H_6–EtOAc eluates were then chromatographed on a silica gel 60 column without activation The C_6H_6 eluate yielded a yellow precipitate identified as naringenin (5,7,4′-trihydroxyflavanone) C_6H_6–EtOAc eluates yielded galangin 3-methyl ether, gnaphalin (5,7-dihydroxy-3,8-dimethoxyflavone), isognaphalin (5,8-di-hydroxy-3,7-dimethoxyflavone), 5,7,8-trihydroxy-3-methoxy-flavone, 5-hydroxy-3,8 dimethoxy 7-(3-methyl-2,3-epoxybutoxy) flavone and 5-hydroxy-7-(3-methyl-2,3-epoxybutoxy)flavanone

The Me_2CO extract was evaporated to dryness and passed through a column packed with polyclar powder The $CHCl_3$–MeOH eluate was concd and run on 1 DPC (Whatman No 3) in 15% HOAc giving bands corresponding tamarixetin (3,3′,5,7-tetrahydroxy-4′-methoxyflavone) and isochlorogenic acid The MeOH–$CHCl_3$ eluate was passed through another polyclar column and yielded 4,2′,4′-trihydroxy-6′-methoxy-chalcone The MeOH eluates gave caffeic acid, chlorogenic acid and 7,4′-dihydroxy-5-methoxyflavanone [5]

The MeOH extract was passed through a polyclar column packed in $CHCl_3$ and eluted with $CHCl_3$–MeOH giving chrysoeriol (5,7,4′-trihydroxy-3-methoxyflavone) and 4′-hydroxy-5-methoxy-7-(3-methyl-2,3-epoxybutoxy)flavone

Gnaphalin 5,7-Dihydroxy-3,8-dimethoxyflavone (40 mg), yellow crystals, mp 174–175° UV λ_{max}^{MeOH} nm 240, 275, 358, NaOMe 246, 282, 330, 390; $AlCl_3$ 281, 330, 395, $AlCl_3$–HCl 281, 330, 390, NaOAc 284, 334, 370; NaOAc–H_3BO_3 276, 363 ^1H NMR ($CDCl_3$) δ7 5 and 8 2 (5H, A_2B_3 system, B ring), 6 38 (1H, *s*, H-6), 4 05 (6H, *s*, OMe), 3 95 (3H, *s*, OMe) [7]

Isognaphalin 5,8-Dihydroxy-3,7-dimethoxyflavone (30 mg), white–yellow crystals, mp 213–215° UV λ_{max}^{MeOH} nm 242, 277, 365, NaOMe 282, 367, $AlCl_3$ 250 sh, 284, 300 sh, 335, 405, $AlCl_3$–HCl 250, 284, 300 sh, 338, 400; NaOAc 276, 365, NaOAc–H_3BO_3 275, 365 ^1H NMR ($CDCl_3$) δ7 5 and 8 2 (5H, A_2B_3 system, B ring), 6 40 (1H, *s*, H-6), 4 00 (3H, *s*, OMe), 3 80 (3H, *s*, OMe) [7]

5,7,8-Trihydroxy-3-methoxyflavone Yield 60 mg Orange

powder, mp > 290° UV λ_{max}^{MeOH} nm 244, 279, 368, NaOMe 266, 375 (dec), $AlCl_3$ 235, 320, 370; $AlCl_3$–HCl 235, 306, 369, 408 sh, NaOAc 265 (dec) ^{1}H NMR ($CDCl_3$): δ7 5 and 8 2 (5H, A_2B_3 system, B ring), δ6 38 (1H, *s*, H-6), δ3 85 (3H, *s*, OMe) MS *m/z* (rel int) 300 $[M]^+$ corresponding to $C_{16}H_{12}O_6$ (14 75%), 284 $[M-Me]^+$ (100%), 266 (16%), 253 (19%), 226 (5%), 171 (28%), 124 (14%), 77 (37 7%)

5-Hydroxy-3,8-dimethoxy-7-(3-methyl-2,3-epoxybutoxy)flavone Yield 20 mg UV λ_{max}^{MeOH} nm 248 sh, 275, 360 sh, NaOMe 283, 364, $AlCl_3$ 280, 320 sh, $AlCl_3$ 279, 321, NaOAc 275, 360 sh, NaOAc–H_3PO_3 unmod ^{1}H NMR (C_6D_6) δ7 6 and 8 2 (5H, A_2B_3 system, B ring), 6 35 (1H, *s*, H-6), 4 45 (2H, *m*, CH_2), 4 45 (3H, *s*, OMe), 4 35 (3H, *s*, OMe), 3 40 (1H, *m*, >CH) 1 1 (6H, *d*, gem di Me) MS *m/z* 398 $[M]^+$ corresponding to $C_{22}H_{23}O_7$ (3%), 298 $[M-R-Me]^+$ (3%), 253 (4%), 225 (3%), 206 (8%), 191 (5%), 167 (9%), 152 (7%), 149 (45%), 119 (10%), 85 (50%), 83 (52%), 67 (14%), 57 (100%), 43 (46%)

5-Hydroxy-7-(3-methyl-2,3-epoxybutoxy)flavanone UV λ_{max}^{MeOH} nm 283, 330 sh, NaOMe 288, 329, $AlCl_3$ 288, 385 sh, $AlCl_3$–HCl 294, 365, NaOAc 282, 327, NaOAc–H_3BO_3 unmod ^{1}H NMR ($CDCl_3$) δ7 50 and 8 1 (5H, A_2B_3 system, B ring), 6 05 (2H, *s*, H-6 and H-8), 5 30 (1H, *dd*, H-2), 4 40 (2H, *m*, CH_2), 3 30 (1H, *m*, >CH), 2 90 (2H, *m*, H-3 *trans* and H-3 *cis*), 1 1 (6H, *d*, gem di Me) MS *m/z* 340 $[M]^+$ ($C_{20}H_{20}O_5$, 10%), 279, 269, 255 $[M-R]^+$ (15%), 179, 167

Naringenin 5-methyl ether UV fluorescent blue–green, UV λ_{max}^{MeOH} nm 281, 321 sh, NaOMe 275, 322 sh, 380; $AlCl_3$ unmod, $AlCl_3$–HCl unmod, NaOAc 279, 325, NaOAc–H_3BO_3 283, 328 sh [7, 8] ^{1}H NMR (DMSO-d_6) δ7 0 (4H, *dd*, typical 4′ substituted B ring), 6 32 (2H, *s*, H-6 and H-8), 5 30 (1H, *dd*, H-2), 3 95 (3H, *s*, OMe), 2 90 (2H, *m*, H-3 *trans* and H-3 *cis*)

4′-Hydroxy-5-methoxy-7-(3-methyl-2,3-epoxybutoxy)flavone UV λ_{max}^{MeOH} nm 259 sh, 285, 342, NaOMe 243, 280, 388, $AlCl_3$ 240 sh, 297, 397, $AlCl_3$–HCl 240 sh, 297, 397, NaOAc 255 sh 284, 339, NaOAc–H_3BO_3 254 sh, 284, 340 ^{1}H NMR (DMSO-d_6) δ7 3 (4H, *dd*, typical 4′ substituted B ring), 6 65 (1H, *d*, H-8), 6 4 (1H, *d*, H-6), 6 25 (1H, *s*, H-3), 4 35 (2H, *m*, CH_2), 3 90 (3H, *s*, OMe), 3 30 (1H, *m*, >CH), 1 00 (6H, *d*, gem di Me) MS *m/z* 368 $[M]^+$ corresponding to $C_{21}H_{20}O_6$ (4 8%), 284 $[M-R_1]^+$ (14%), 278, 264, 236, 185, 137, 85, 83, 57 (100%) The other flavonoids were identified by UV spectral shifts [8] and comparison with authentic samples Caffeic acid, chlorogenic acid and isochlorogenic acid were identified by HPLC using an RP 18 column eluted with MeOH–0 1 N KH_2PO_4 (33 67) by comparison with authentic samples

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REFERENCES

1 Parodi, D (1886) *Plantas usuales del Paraguay, de Corrientes y de Misiones* P Coni, Buenos Aires, Argentina
2 Paccard, E (1905) *Plantas medicinales de las Repúblicas Oriental y Argentina* A Ramos, Montevideo, Uruguay
3 Hieronymus, J (1882) *Bol Acad Nac Cienc Córdoba* **4**, 199
4 Ferraro, G E, Norbedo, C and Coussio, J D (1981) *Phytochemistry* **20**, 2053
5 Norbedo, C, Ferraro, G E and Coussio, J D (1982) *Lloydia* **45**, 635
6 Heywood, V, Harborne, J and Turner, B (1977) *The Biology and Chemistry of the Compositae* Academic Press, London
7 Wagner, H, Maurer, G, Farkas, L, Hansel, R and Ohlendorf, D (1971) *Chem Ber* **104**, 2381
8 Mabry, T J, Markham, D and Thomas, M (1970) *The Systematic Identification of Flavonoids* Springer, New York
9 Harborne, J B, Mabry, T J and Mabry, H (eds) (1975) *The Flavonoids* Academic Press, New York