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FLAVONOIDS FROM *CENTAUREA CLEMENTEI*[1]

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The genus *Centaurea* (Compositae) encompasses one of the greatest number of species (approximately 700) within the tribe Cynareae. The *Centaurea* are found mainly in the Mediterranean area, southwest Asia, some regions of Africa, North America, and South America (Chile). This genus has been studied from the point of view of its sesquiterpene lactone contents (1,2) and, to a lesser extent, its flavonoid content.

Centaurea clementei DC. is a perennial plant that grows in the calcareous soils in the southwestern part of the Iberian Peninsula (3). Preliminary phytochemical studies revealed the presence of sesquiterpene lactones of the guayane type (4), some of which show the infrequent methyloxetane ring (5). Further tlc screening revealed the presence of flavonoid compounds.

From the ethanolic extract of the aerial parts of *C. clementei*, three flavones and one flavonol were isolated. Negletein (**1**) is reported here for the first time as an apparently free natural product, although it was previously obtained by the hydrolysis of the flavone glycosides, neglectin (6) and palustrin (7). The other flavonoids were identified as 4′,7-di-*O*-methyl scutellarein (**2**) (8), hispidulin (**3**) (9), and isokaempferide (**4**) (10), by uv, ir, ms, ^{1}H-nmr and ^{13}C-nmr data.

Negletein was obtained in a very small amount, as yellow crystals $C_{16}H_{12}O_5$, 284.0701 (M^+, 100%). Its uv spectrum displayed λ max (MeOH) 271 and 318 nm and using standard shift reagent the following structural features were deduced: (a) the absence of a shift of band II (271 nm), by addition of NaOAc, indicated that the C-7 is substituted by a OMe; (b) a 20 nm bathochromic shift of band I (318 nm) with $AlCl_3$ and 19 nm with $AlCl_3$+HCl were consistent with the presence of a free OH at C-5.

The ^{1}H-nmr spectrum of negletein showed the presence of two singlets at δ 6.68 (1H) and δ 6.63 (1H), which were assigned to the C-8 (or C-6) proton of the A-ring and C-3 proton of the γ-pyrone ring, respectively. The multiplet centered at δ 7.70 corresponded to the five protons of the B-ring, and the singlet (3H) at δ 4.04 was attributed to the O-methyl group at C-7. Furthermore, a singlet (1H) at δ 13.50 showed the presence of a hydroxyl at the C-5 position because the proton of a 5-hydroxy group next to a 4-carbonyl typically gives rise to a sharp signal, at very low field, consistent with the strong hydrogen bonding between the two groups (11).

From the above ^{1}H-nmr data, it is very difficult to establish unambiguously the substitution pattern on the A-ring because H-6 and H-8 have similar chemical shifts. To discern the position in which the second hydroxyl is found (C-6 or C-8), we followed the rule of Mears and Mabry (12), which uses the different shifts of band I of the uv, in $AlCl_3$+HCl, with respect to the spectrum carried out in MeOH. As the bathochromic shift of **1** was 19 nm, the hydroxyl must be on C-6. (If it were on C-8, it would be around 40.50 nm.)

The mass spectrum of **1** showed a peak at *m/z*, 249.0701 corresponding to the molecular ion, and fragments at 269.0398, 266, 241.0475, etc. These were explained as shown in Scheme 1.

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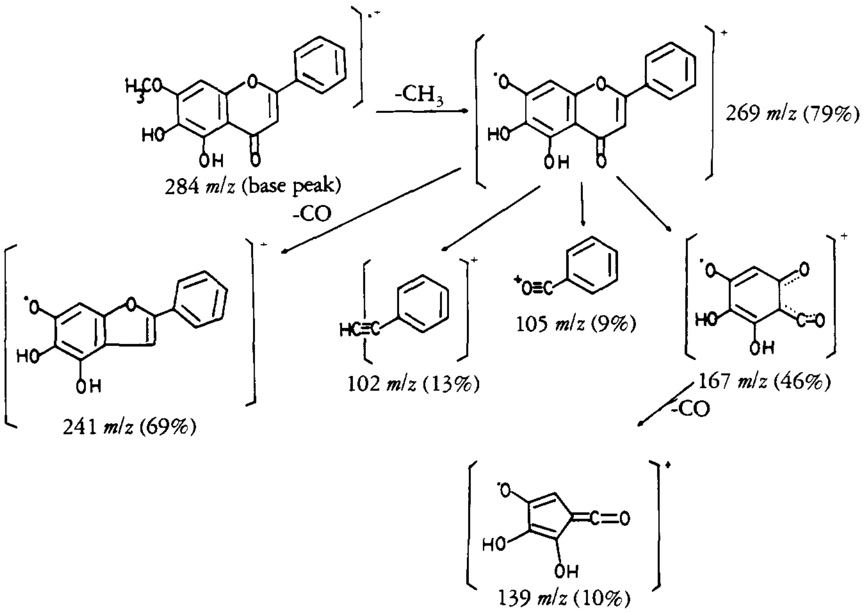
[1] Part 5 in the series "Structure and Chemistry of Secondary Metabolites from Compositae." For part 4, see I. González Collado, F.A. Macías, G.M. Massanet, and F. Rodríguez Luis, *Phytochemistry*, (in press).



SCHEME 1. Proposed Mass Fragmentation of **1**

The ^{13}C-nmr spectrum (Table 1) is consistent with structure **1**, and the assignments were based on the work of Mabry *et al.* (13). A doublet at δ 94.4 was assignable to the unsubstituted C-8; this value is between 90.0 and 95.0, the characteristic zone of this carbon when not substituted (14). A singlet assignable to C-7 appeared at δ 157.3, a typical value of C-7 when substituted by non-hydroxylic oxygenated functions.

Structure **1** for negletein was confirmed by the formation of the diacetyled derivative, **1a**, in the ir spectrum of which the hydroxyl bands have disappeared. In the ^{1}H-nmr spectrum, the presence of two acetate singlets was observed at δ 2.50 (C_5-OCOCH_3) and

TABLE 1. ^{13}C nmr of Flavonoids **1, 3, 4** from *Centaurea clementei*[a]

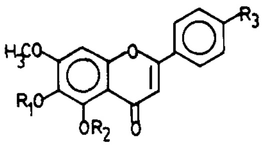
Carbon number	**1** (DMSO-d_6)	**3** (DMSO-d_6)	**4** (DMSO-d_6)
C-2	163.20 s	163.70 s	157.32 s
C-3	104.70 d	102.40 d	139.39 s
C-4	182.20 s	182.10 s	179.61 s
C-5	152.60 s	152.80 s[b]	163.06 s
C-6	130.70 s	131.30 s	100.73 d
C-7	157.30 s	157.10 s	167.06 s
C-8	94.40 d	94.20 d	95.72 d
C-9	152.60 s	152.40 s[b]	158.32 s
C-10	104.70 s	104.10 s	105.71 s
C-1′	131.40 s	121.00 s	122.42 s
C-2′	126.40 d	128.40 d	131.90 d
C-3′	129.10 d	115.90 d	117.54 d
C-4′	132.00 d	161.00 s	162.07 s
C-5′	129.10 d	115.90 d	117.54 d
C-6′	126.00 d	128.40 d	131.90 d
-OCH$_3$	60.00 c	59.90 c	61.53 c

[a] Determined on a 99.55 MHz instrument with TMS as internal standard. Signals were assigned by means of off-resonance decoupled spectra.

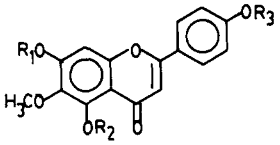
[b] Assignments may be interchanged.

2.39 (C_6-OCOCH_3) at the same time as H-8 was paramagnetically shifted to 7.30 ppm.

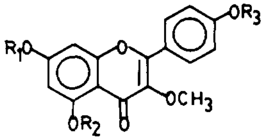
4′,7-Di-*O*-methyl scutellarein was characterized by spectroscopic study of **2** and **2a**. Hispidulin (**3**) has been known since 1964, when it was isolated from *Ambrosia hispida* Pursh (9). We identified it by spectral data, as well as by its 4′,7-diacetate **3a**, triacetate **3b**, and trimethyl ether **3c**. The isokaemferide (**4**) was identified by spectroscopic study and by the formation of its triacetate **4a**.



1 $R_1=R_2=R_3=H$
1a $R_1=R_2=Ac; R_3=H$
2 $R_1=R_2=H; R_3=OCH_3$
2a $R_1=R_2=Ac; R_3=OCH_3$



3 $R_1=R_2=R_3=H$
3a $R_1=R_2=R_3=Ac$
3b $R_1=H; R_2=R_3=Ac$
3c $R_1=R_3=CH_3; R_2=H$



4 $R_1=R_2=R_3=H$
4a $R_1=R_2=R_3=Ac$

EXPERIMENTAL

PLANT MATERIAL.—Samples of *C. clementei* were collected in July 1981, from Peñón de Algámitas, in Sevilla, Spain. A voucher specimen (SEV #55499) is deposited at the Botanic Department Herbarium, Faculty of Pharmacy, University of Sevilla.

EXTRACTION.—Approximately 2 kg of the dried aerial parts were exhaustively extracted with EtOH in Soxhlet extractors. The alcoholic extract was concentrated under reduced pressure, and the resulting product was chromatographed on a silica gel column. Elution was done with petroleum ether and increasing quantities of EtOAc and 51 fractions (50-60 ml each) were collected to yield 30 mg **1**, 50 mg **2**, 50 mg **3**, and 200 mg **4**. Compounds **2-4** were identified by uv, ir, ms, ^{1}H-nmr, and ^{13}C-nmr data.

NEGLETEIN (**1**).—Mp 238-239°; ir ν max (KBr) 3500, 1650, 1600, 1260, 1015, 855, 805, 770 cm^{-1}; ^{1}H nmr (60 MHz, $CDCl_3$) δ, 13.50 (s, 1H, C_5-O*H*), 7.70 (m, 5H, B-ring), 6.68 (s, 1H, H-8), 6.63 (s, 1H, H-3), and 4.04 (s, 3H, -OC*H*$_3$). Ms *m/z* (rel. int.), 284.0701 [M^+] (base), ($C_{16}H_{12}O_5$), 269.0398 $[M-15]^+$ (79), 266 $[M-18]^+$ (51), 241.0475 $[M-15-CO]^+$ (69), 167 (4.5), 139 (10), 105 (9), and 102 (13). Uv λ max (MeOH) 249 (sh), 271, 318; ($AlCl_3$) 254 (sh), 283, 338; ($AlCl_3$+HCl) 254 (sh), 283, 337; (NaOMe) 243 (sh), 268, 367; (NaOAc) 268, 368; (NaOAc+H_3BO_3) 272, 322.

DIACETATE (**1a**).—Acetylation of **1** with 1 ml Ac_2O-pyridine (3:1), 24 h, room temperature. After purification by preparative tlc (petroleum ether-EtOAc, 7:3), afforded **1a**, mp 135-137° (EtOAc). ^{1}H nmr (60 MHz, $CDCl_3$) δ, 7.70 (m, 5H, B-ring), 7.30 (s, 1H, H-8), 6.64 (s, 1H, H-3), 3.89 (s, 3H, -OC*H*$_3$), 2.50 (s, 3H, C_5-OAc) and 2.39 (s, 3H, C_6-OAc). Ir ν max (KBr) 2850, 1750, 1620, 1230, 1200, 1020, 880, 850, 760 cm^{-1}.

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LITERATURE CITED

1. A.G. González, J. Bermejo, and G.M. Massanet, *Rev. Latinoamer. Quim.*, **8**, 176 (1977).
2. N.H. Fischer, E.J. Olivier, and H.D. Fischer, in: *Progress in the Chemistry of Organic Natural Products*, Berlin, Springer-Verlag, 1979, pp. 130-131, 201, and 315.
3. O. Polunin and B.E. Senythies, *Guía de*

campo de las flores de España, Barcelona, Editorial Omega S.A., 1977, p. 393.
4. A.G. González, J. Bermejo, G.M. Massanet, and J. Redondo, unpublished results, in J. Redondo Thesis, Universidad de la Laguna, Tenerife, Spain (1974).
5. G.M. Massanet, I.G. Collado, F.A. Macías, F. Bohlman, and J. Jakupovic, *Tetrahedron Lett.*, **24**, 1641 (1983).
6. T.V. Zinchenco, *Farm. Zh.*, **25**, 81 (1970).
7. T.V. Zinchenco, *Khim. Prir. Soedim.*, **6**, 266 (1970).
8. L. Farkas, G. Mezey-Vandor, and M. Nogradi, *Chem. Ber.*, **104**, 2646 (1971).
9. W. Herz and Y. Sumi, *J. Org. Chem.*, **29**, 3438 (1964).
10. L.E. Urbartsch, J.D. Bacon, and T.J. Mabry, *Phytochemistry*, **19**, 929 (1980).
11. T.J. Mabry, K.R. Markham, and M.B. Thomas, *The Systematic Identification of Flavonoids*, Berlin, Springer-Verlag, 1970, p. 262.
12. J.A. Mears and T.J. Mabry, *Phytochemistry*, **11**, 411 (1972).
13. T.J. Mabry, K.R. Markham, and V.M. Chari, *The Flavonoids,* London, Chapman and Hall, 1982 pp. 51-134.
14. V.M. Chari, S. Ahmad, and B.G. Osterdahl, *Z. Naturforsch*, **33b**, 1547 (1978).

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