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FLAVONE-*C*-GLYCOSIDES IN *HELENIUM* SPECIES

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Plant. Helenium alternifolium (Spreng) Cabrera *Source* Collected by P R. Legname in 1966, near Tucuman, Argentina *H autumnale* L *Sources* (1) (whole plant) Collected by Dr. McDaniel in Alabama, September 1967 (McDaniel voucher 9867) (2) (whole plant). Collected by R Lazor in September 1968, 13 miles south of Moultrie, Georgia (Godfrey-Lazor voucher 1185 on deposit in herbarium of Florida State University) *Previous work on H alternifolium from various sources* 3′,6-Dimethoxy-4′,5,7-trihydroxyflavone,[1] sesquiterpene lactones [1] The collection used in the present work gave[1] brevilin A, linifolin A, tenulin and an unidentified lactone $C_{19}H_{26}O_5$ *On H autumnale from various sources* Sesquiterpene lactones [2,3] The collections used in the present work gave[2] helenalin

Compounds isolated Saponaretin, iso-orientin, vitexin and orientin were isolated from the methanolic extracts of the plants by methods described previously[4] and identified by direct comparison with authentic material by mixed m p , co-chromatography (TLC, 3 solvents), UV and IR analysis

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Key Word Index—Helenium spp , Compositae, Glycoflavones, saponaretin, orientin

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THE CONSTITUENTS OF *HELICHRYSUM STOECHAS*

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Abstract—The aerial parts of *Helichrysum stoechas* (L) D C have yielded ursolic and oleanolic acids, uvaol and erythrodiol, β-sitosterol and stigmasterol (both free and combined as the β-glucoside), fatty acids, the acetophenone (I), a related alcohol (II) and a new chroman (III)

INTRODUCTION

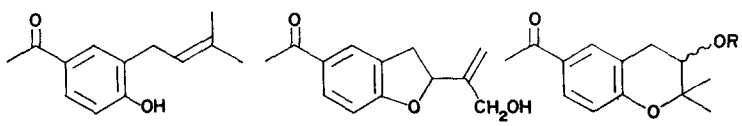
Helichrysum stoechas is a woody shrub characteristic of the Mediterranean area [1] Up to the present, chemical studies have only been carried out on the essential oils [2–5] In this communication, we report various products isolated from the aerial parts of this plant.

RESULTS

The triterpenic fraction consists of ursolic and oleanolic acids and the triterpene alcohols uvaol and erythrodiol, the high content of ursolic acid (3 2% on dry plant) is unusual The sole steroidal components are β-sitosterol and stigmasterol

GLC of the fatty acids (as their methyl esters) showed the presence of linoleic (29%), palmitic (24 5%), linolenic (23%) and oleic (9·3%) acids, with minor quantities of the remaining acids of the even-numbered series Components of the odd-numbered series are only present in small proportion (2 6% of the total amount) and within this the C_{13}, C_{15}, C_{17}, C_{21} and C_{23} components, with one or two unsaturations, have been detected

Three other substances, with strong UV absorption have also been isolated. The m p and the UV, IR, NMR and mass spectra show that one of these is 4-hydroxy-3[isopentent-2-yl]acetophenone (I), recently isolated for the first time from *Helianthella uniflora* (Compositae) [6]



(I) (II) (III) R = H (IV) R = Ac

A second substance is an optically active liquid ($n_D^{13.5}$ 1·5785; $[\alpha]_D^{28}$ −61·2°) with molecular formula $C_{13}H_{14}O_3$, the spectral data and the reactivity of this compound show it has structure II Two new esters, the alcoholic part of which corresponds to structure II, have recently been isolated [7] This is the first time that the free alcohol is reported in nature.

The third UV-absorbing component has been isolated in small amount (0 009% on dry plant) The NMR spectrum of this substance is extremely simple τ 8·62 (6H, s, gem dimethyl), 7 47 (3H, s, CH_3—CO—Ph), 7·0 (2H, m, benzylic), 6·12 (1H, q, *J* 5 6 cps, —CHOH—), 3·14 (1H, d, *J* 9 3 cps, aromatic) and 2 23 (2H, s,d, aromatic) It points to structure III (2,2-dimethyl-3-hydroxy-6-acetylchroman) for this product The preparation of a monoacetate (IV) and the interpretation[8] of the mass spectrum of this last compound confirm this asignment

Compound III has been suggested[6] as a possible intermediate in the biosynthesis of some natural chromenes, though this is the first time that it has been actually isolated We have also observed that when a chloroform solution of I is left several days in sunlight, various products, among which III can be identified, are formed

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Finally, we have isolated a substance which is practically insoluble in the ordinary solvents [m.p. 283–284° (decomp), $[\alpha]_D^{25}$ −46°] and which forms a tetraacetate (m.p 168–169°, $[\alpha]_D^{25}$ −26 2°) It gives a single spot on chromatography on silica and alumina plates impregnated with $AgNO_3$ The NMR spectrum (60 MHz) of the tetraacetate indicates it to be a monoglycoside of a sterol In fact, on prolonged acid hydrolysis, it yields D-glucose and a sterol that, on acetylation, is resolved into two components, β-sitosterol and stigmasterol The NMR spectrum (100 MHz) of the tetraacetyl glucoside shows that the product is a mixture of β-sitosterol and stigmasterol β-D-glucopyranosides.

EXPERIMENTAL

M ps were observed on a Kofler hot stage and are uncorrected The UV spectra were in EtOH and the NMR spectra were recorded in $CDCl_3$ with tetramethylsilane as internal reference The GLC of the fatty acid methyl esters was carried out on a Perkin–Elmer Model F-20 using standard conditions

Extraction of the plants Air dried and finely powdered aerial parts of *H stoechas* (5 7 kg) were extracted in a Soxhlet with MeOH for 60 hr The crystalline precipitate (175 g) was decolorized (active charcoal) and collected as a white powder (168 g) m p 286–289° (from CH_3OH–HO, $[\alpha]^{25}{}_{2D}$ +61 0 (*c* 0 37, pyridine) The physical constants and spectroscopic data obtained with this product, with the acetyl, methyl ester and acetyl-methyl ester derivatives, and with the $LiAlH_4$ reduction product of the last compound are in complete agreement with those reported in the literature[9] for ursolic acid and its corresponding derivatives

Separation of the components Vacuum distillation of the methanolic extract leaves a syrupy residue (1 04 kg) A portion of the syrup (50 g) was chromatographed on a silica gel column (2 kg), giving the following fractions (a) A mixture of hydrocarbons (1 5 g) eluted with benzene (discarded), (b) a mixture of sterols and triterpene alcohols (7 g) eluted with $CHCl_3$, (c) a complex mixture (36 g) containing triterpene acids, fatty acids and three substances absorbing in the UV, eluted with $CHCl_3$–CH_3OH (9 1), and (d) sterol glycosides (1 76 g) eluted with $CHCl_3$–CH_3OH (7 3)

Acetylation of fraction (b) and column chromatography on silica gel allows the separation of two fractions (b_1 and b_2) Preparative TLC on silica gel plates (40% $AgNO_3$) of b_1 yields two substances that are identified (m p , mixed m p , $[\alpha]_D$, MS, IR, NMR and mobility on silica gel plates impregnated with $AgNO_3$) as β-sitosterol and stigmasterol acetates respectively Fractional crystallization of b_2 from MeOH yields uvaol and erythrodiol diacetates, identity being established by comparison with authentic samples

Fraction (c) was chromatographed on silica gel $CHCl_3$–MeOH (9 1) elutes first a mixture (10 9 g) of I + III, followed by material containing II, triterpene and fatty acids The portion containing I + III was resolved by preparative TLC on silica gel plates, I is the less polar of the two

The material containing II and the triterpene and fatty acids (26 g) was chromatographed on neutral alumina (grade III, 400 g) Elution with $CHCl_3$–CH_3OH (97 3) affords compound II (8 5 g); while a mixture of the triterpene and fatty acids is obtained eluting with $CHCl_3$–CH_3OH (3 2) The mixture was digested with $CHCl_3$, the fatty acids (2 g) being completely solubilized while the triterpene acids (15 5 g) remained insoluble The fatty acids were methylated by treatment with CH_2N_2 and the mixture analysed by GLC (see Results) The mixture of triterpene acids was acetylated (Ac_2O/Py) Fractional crystallization from methanol yields the acetyl derivatives of ursolic acid (14 2 g) and oleanolic acid (0 7 g), identification being accomplished by its physical and spectroscopic data and by comparison with authentic samples

Compound I 4-Hydroxy-3 [isopenten (2)-yl]acetophenone M p 92–93° (from acetone–hexane), $[\alpha]_D^{22}$ 0 00° (*c* 1 56, $CHCl_3$), UV λ_{max}^{EtOH} 226 nm (ε 16,200) and 280 nm (ε 14,500), undergoing a bathochromic shift in basic medium [λ_{max}^{EtOH} 246 nm (ε 8100) and 343 nm (ε 27,800)], IR (Nujol) 3160, 1650, 1586, 1510, 1290, 1250, 828 cm^{-1}, NMR (τ units) 8 24 (6H, s, isopropenyl), 7 42 (3H, s, CH_3—CO—Ph), 6 59 (2H, dd, *J* 7 3 cps, benzylic), 4 64 (1H, t, *J* 7 3 cps, vinylic), 3 06 (1H, d, *J* 9 3 cps, aromatic) and 2 21 (2H, s, d, aromatic) Mass spectrum *m/e* 204 (M^+, 31%), 189 (M − CH_3, 44%), 187 (M-17, 22%), 161 (M − $COCH_3$, 13%), 149 (M − C_4H_7, 50%), 133 (M − CH_3 − C_4H_7 − H, 30%), 91 (13%), 77 (15%) and 43 (100%) (Found C, 76 14, H, 7 84 Calc for $C_{13}H_{16}O_2$ C, 76 44, H, 7 90%)

Compound II Thick liquid, $n_D^{13\ 5}$ 1 5785, $[\alpha]_D^{28}$ −61 2° (*c* 2 01, $CHCl_3$), UV λ_{max}^{EtOH} 227 nm (ε 11,100) and 280 nm (ε 12,400), IR (film) 3420, 3100, 3080, 3010, 1665, 1600, 1590, 1165, 1120 and 820 cm^{-1}, NMR (τ units) 7 5 (3H, s, CH_3—CO—Ph), 6 7 (2H, m, benzylic), 5 75 (2H, s, —CH_2OH), 4 7 (2H, broad singlet, >C=CH_2), 4 6 (1H, t, —O—CH—C=C—), 3 15 (1H, d, *J* 9 cps, aromatic) and 2 20 (2H, s, d, aromatic) Mass spectrum *m/e* 218 (M^+) (Found C, 71 11, H, 6 45 Calc for $C_{13}H_{14}O_3$ C, 71 56, H, 6 12%)

Compound III Found C, 70 78, H, 7 27 Calc for $C_{13}H_{16}O_3$ C, 70 89, H, 7 32%

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Acetyl derivative of III (IV) Compound III (30 mg) was acetylated with Ac_2O/Py and the product crystallized from CH_3OH, m p 121–122°, λ_{max}^{EtOH} 222 nm (ϵ 8100) and 274 nm (ϵ 10,400), NMR τ4 90 (1H, t, J 5 6 cps, —CH—OAc). Mass spectrum *m/e* 262 (M^+, 3%), 202 (M − AcOH, 21%), 187 (M − AcOH − CH_3, 100%), 159 (M − AcOH − CH_3CO, 9%), 149 (12%), 107 (8%), 91 (7%) and 77 (8%)

Transformation I→III Compound I (30 mg) was dissolved in $CHCl_3$ (5 ml) and the solution was left (8 days) exposed to sunlight Analysis by TLC of the reaction product indicates the presence of six new compounds besides the starting material I The most polar of these components, separated by preparative TLC, appears to be identical to compound III (by TLC comparison in various systems)

Sterol glycosides The product from fraction (d) behaves as a single substance in all the chromatographic systems tested m p 283–284° (decomp) (from $CHCl_3$–CH_3OH), $[\alpha]_D^{26}$ −46 1° (*c* 0 37, pyridine) The tetraacetyl derivative (obtained in the usual manner) crystallizes from CH_3OH, m p 168–169°, $[\alpha]_D^2$ −26 2° (*c* 0 52, $CHCl_3$) (Found C, 69 70, H, 9 24 Cal for $C_{43}H_{68}O_{10}$ C, 69 32, H, 9 20%)

Hydrolysis of the glycoside The glycoside (150 mg) was refluxed for 10 hr in EtOH (50 ml), H_2O (10 ml) and conc HCl (5 ml) The solution was diluted with H_2O and extracted with $CHCl_3$ The aqueous phase was desalted (passing it through ion exchange resins) and evaporated, the residue (15 mg) was identified as D-glucose (paper chromatography of the free sugar and of its 2,4-dinitrophenylhydrazone derivative on TLC)

Evaporation of the $CHCl_3$ extract yields 92 mg of a crystalline product that, once acetylated (Ac_2O/Py), can be separated (SiO_2 40% $AgNO_3$ preparative TLC plates eluted with $CHCl_3$) in two components present in a 1 1 ratio Pure samples of these two components are identical (m p , mixed m p , $[\alpha]_D$, IR, NMR and mass spectra) with β-sitosterol and stigmasterol acetates

Key Word Index—Helichrysum stoechas, Compositae, triterpenes, phytosterols, chroman, 4-hydroxy-3-isopentylacetophenone

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COMPOSITION OF PUNJAB COSTUS ROOT OIL

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Plant. Saussurea lappa Clarke. *Source.* Lahaul and Spiti areas of Punjab, India [1] *Uses* In the indigenous system of medicine,[2] roots are reported tonic, stomachic, carminative, stimulant and useful in asthma, cough and cholera. It is said to have remarkable effect in controlling bronchial asthma, especially of vagatonic type [3, 4] *Previous work.* Costus root oil obtained from plants of Kashmir (India) origin has been studied critically [5–7] The

* This work was presented in XX Annual convention of AsoVAC, held at Caracus (Venezuela) in May 1970

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