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METHYLENE-*BIS*-2H-PYRAN-2-ONES AND PHENOLIC CONSTITUENTS FROM THE ROOT OF *HELICHRYSUM ARENARIUM*

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Key Word Index—*Helichrysum arenarium*; Compositae; methylene-*bis*-4-hydroxy-2H-pyran-2-ones; helipyrone; norhelipyrone; bisnorhelipyrone; flavonoids.

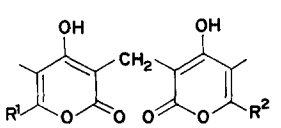
Abstract—In the polar fraction of the extract from *Helichrysum arenarium* roots, the methylene-*bis*-4-hydroxy-2H-pyran-2-one derivatives, helipyrone and two new homologues norhelipyrine and bisnorhelipyrone, were identified. Two antibacterial glycosides, $C_{19}H_{24}O_9$ and $C_{30}H_{44}O_{14}$, and the flavonoids galangin, naringenin and kampferol-3-glucoside were also found.

INTRODUCTION

Several compounds containing alkyl substituted derivatives of 4-hydroxy-2H-pyran-2-one bound to phenols or enols by means of a methylene bridge have recently been isolated from several related members of the compositae [1–4]. One type of these compounds is represented by arenol and homoarenol [4] which were isolated from blossoms of *Helichrysum arenarium* and exhibit a remarkable antimicrobial effect. In arenol and homoarenol, 4-hydroxy-2H-pyran-2-one is substituted at position 6 by a methyl or ethyl group. In the roots of the same plant, we have now found the methylene-*bis*-4-hydroxy-2H-pyran-2-one derivatives helipyrone (observed earlier in *H. italicum* [1]) and two new analogues exhibiting substitution at position 6 by a methyl or ethyl group, similar to that found in arenol and homoarenol.

RESULTS AND DISCUSSION

The procedure described in the Experimental afforded two extracts A and B. The components of extract A were isolated in a pure state after acetylation and column chromatography yielded two



(1) $R^1 = R^2 = C_2H_5$
(2) $R^1 = Me$; $R^2 = C_2H_5$
(3) $R^1 = R^2 = Me$

compounds, $C_{21}H_{24}O_8$ and $C_{19}H_{20}O_8$ (MS). Preparative TLC of a smaller fraction gave a small amount of a compound $C_{20}H_{22}O_8$.

The PMR spectra of the compounds $C_{21}H_{24}O_8$ and $C_{19}H_{20}O_8$ both indicated a symmetrical molecule. With respect to the number of protons, obtained from MS, each signal must correspond to two symmetrically equivalent groups of protons. The spectrum of compound $C_{21}H_{24}O_8$ exhibited a triplet at δ 1·5 and a quartet at δ 2·48, attributable to two ethyl groups bound to a quaternary sp^2 carbon atom, a singlet at δ 1·71 corresponding to two methyl groups on sp^2 carbon atoms, a singlet at δ 2·26 corresponding to two acetoxy groups, and finally a singlet at δ 3·50 which was assigned to a methylene group between two sp^2 carbon atoms. The PMR spectrum of compound $C_{19}H_{20}O_8$ differed from that of compound $C_{21}H_{24}O_8$ by the presence of a singlet at δ 2·22 corresponding to two methyl groups bound to sp^2 carbon atoms instead of ethyl groups. The remaining signals were the same.

The IR spectra of all the three acetyl derivatives exhibit bands at about 1765 and 1708 cm^{-1} indicative of the presence of a further carbonyl group (in addition to the acetyl group).

On the basis of the above data, some physical constants, and UV spectra, compound $C_{21}H_{24}O_8$

was identified as the diacetyl derivative of helipyrone (**1**). The product obtained on deacetylation exhibited properties identical with those of helipyrone occurring in *H. italicum* [1].

In compound $C_{19}H_{20}O_8$, the two ethyl groups are replaced by two methyl groups; deacetylation leads to compound **3** which was named bisnorhelipyrone.

The structure of compound $C_{20}H_{22}O_8$ was assigned on the basis of MS and IR data alone. This compound represents a diacetyl derivative of an asymmetrically substituted helipyrone analogue **2** (methyl and ethyl groups as substituents). Compound **2** was named norhelipyrone. The MS of the diacetyl derivatives of helipyrones **1**–**3** were analogous.

The components of extract B were chromatographed on polyamide. The first fractions (H_2O as eluant) contained an unidentified mixture of sugars. Further fractions (H_2O and 3:1 H_2O–MeOH as eluants) contained a mixture of two glycosides of the empirical formula $C_{19}H_{24}O_9$ and $C_{30}H_{44}O_{14}$ which exhibited antibacterial activity. The final fractions (1:1 H_2O–MeOH and MeOH as eluants) represented mixtures of glycosides and aglycones of flavonoid compounds; kampferol-3-glucoside, naringenin, and galangin were identified as the principal components.

EXPERIMENTAL

M.ps. were determined on a Kofler block. Si gel G was used for TLC. The PMR spectra were recorded at 100 MHz in $CDCl_3$ with TMS as internal standard. MS were obtained using the direct inlet method at 70 eV.

Extraction. Roots of *H. arenarium* L. Moench (1·5 kg) collected along the highway Malacky–Bratislava (Czechoslovakia) in 1972 (sample No. 36/1972) was deposited in the Herbarium of Inst. Org. Chem. and Biochem., Průhonice near Prague, Czechoslovakia, were dried, finely ground, and extracted with petrol. and then with EtOH. The petrol. extract was concentrated and the concentrate distributed between 60% aq EtOH and petrol.; the aq EtOH portion was extracted with $CHCl_3$ to afford extract A. The aq EtOH extract was concentrated and distributed between 60% aq EtOH and C_6H_6; the aq EtOH portion was repeatedly extracted with $CHCl_3$–EtOH (4:1) to afford extract B.

Extract A (3·1 g) was chromatographed on 300 g of Si gel (15% of H_2O) with $CHCl_3$–MeOH (98:2) to afford crystals m.p. 210°–230°. Acetylation (C_5H_5N–Ac_2O) gave per-acetyl compounds which were chromatographed on Si gel using C_6H_6–Et_2O (9:1) as eluant. The first and the last fractions afforded the pure diacetyl derivatives of helipyrone and bisnorhelipyrone, respectively. The middle fractions were rechromatographed using preparative-TLC to furnish the norhelipyrone diacetyl derivative.

Helipyrone diacetate. M.p. 151–152°; λ_{max} 307 nm (log ϵ 4·17); γ_{max} 1768 and 1708 cm^{-1}; MS: M^+ 404 (16, $C_{21}H_{24}O_8$), 362 (22), 321 (20), 320 (100, $C_{17}H_{20}O_6$), 319 (10), 292 (8), 291 (16, $C_{15}H_{15}O_6$), 263 (13, $C_{14}H_{15}O_5$), 245 (13, $C_{14}H_{13}O_4$), 235 (5), 233 (5), 208 (6), 207 (23, $C_{11}H_{11}O_4$), 180 (14, $C_{10}H_{12}O_3$), 179 (8), 167 (15, $C_9H_{11}O_3$), 155 (13, $C_8H_{11}O_3$), 154 (22, $C_8H_{10}O_3$), 151 (7), 139 (11, $C_8H_{11}O_2$), 126 (13, $C_7H_{10}O_2$), 113 (12, $C_6H_9O_2$, 83 (13), 57 (33), 43 (42). PMR: δ 1·15 (6H, *t*, *J* 7·5 Hz, 2 × $C\underline{H}_3$–$C\underline{H}_2$), 1·71 (6H, *bs*, 2 × Me–C=), 2·26 (6H, *s*, 2 × Me–CO–O–), 2·48 (4H, *q*, *J* 7·5 Hz, Me–$C\underline{H}_2$–), 3·50 (2H, *s*, ≥C–$C\underline{H}_2$–C≤).

Helipyrone (**1**). M.p. 217°; λ_{max} 296 nm (log ϵ 4·10); γ_{max} 1683 cm^{-1}, strong H bonding at 2630 to 3100 cm^{-1}; MS: M^+ 320. PMR: δ 1·19 (6H, *t*, *J* 7·5 Hz, 2 × $C\underline{H}_3$–CH_2), 1·95 (6H, *s*, 2 × Me–C=), 2·56 (4H, *q*, *J* 7·5 Hz, 2 × Me–$C\underline{H}_2$–), 3·54 (2H, *s*, ≥C–CH_2–C≤), 11·19 (*s*, 2 × OH).

Norhelipyrone diacetate. M.p. 158–163°; γ_{max} 1765 and 1708 cm^{-1}; MS: M^+ 390 (2), 348 (7), 306 (28), 291 (1), 278 (2), 277 (3), 263 (2), 249 (3), 245 (3), 235 (1), 231 (2), 221 (2), 207 (6), 193 (5), 180 (5), 167 (18), 166 (6), 155 (3), 154 (8), 153 (9), 141 (4), 140 (7), 139 (13), 126 (8), 113 (7), 112 (9), 99 (10), 83 (17), 57 (21), 43 (100).

Norhelipyrone (**2**). M.p. 230–235°, γ_{max} 1684 cm^{-1}, strong H bonding at 2630–3100 cm^{-1}.

Bisnorhelipyrone diacetate. M.p. 170°; λ_{max} 305 nm (log ϵ 4·17); γ_{max} 1765 and 1708 cm^{-1}; MS: M^+ 376 (11, $C_{19}H_{20}O_8$), 334 (18), 293 (14), 292 (85, $C_{15}H_{16}O_6$), 277 (4), 274 (5), 264 (7), 249 (10), 231 (9), 221 (6), 194 (10), 193 (30, $C_{10}H_9O_4$), 166 (19, $C_9H_{10}O_3$), 165 (11), 153 (15), 141 (14), 140 (25, $C_7H_8O_3$), 125 (15, $C_7H_9O_2$), 112 (19, $C_6H_8O_2$), 99 (18, $C_5H_7O_2$), 83 (7), 55 (16), 43 (100). PMR: δ 1·70 (6H, *bs*, 2 × Me–C=), 2·17 (6H, *bs*, 2 × Me–C≤), 2·26 (6H, *s*, 2 × Me–CO–O–), 3·50 (2H, *s*, ≥C–CH_2–≤).

Bisnorphelipyrone (**3**). M.p. 245°, λ_{max} 296 nm (log ϵ 4·12); γ_{max} 1685 cm^{-1}, strong H bonding at 2630 to 3100 cm^{-1}; MS: M^+ 292. PMR: δ 1·93 (6H, *bs*, 2 × Me–C=), 2·22 (6H, *bs*, 2 × Me–C≤), 3·52 (2H, *s*, ≥C–CH_2–C≤), 11·14 (2H, *s*, 2 × –OH).

Extract B (14 g) was chromatographed on 1 kg of polyamide. The column was successively eluted with H_2O, H_2O–MeOH (3:1), H_2O–MeOH (1:1) and MeOH. On the basis of TLC (Si gel G, $CHCl_3$–MeOH (9:1)), the corresponding eluates were combined and processed to afford the following compounds: *Glycoside* $C_{19}H_{24}O_9$. M.p. 184° (MeOH). Optical $[\alpha]_D^{25}$ −4° (*c* 0·92; MeOH). *Glycoside* $C_{30}H_{44}O_{14}$. M.p. 109–110° (MeOH). $[\alpha]_D^{20}$ −48° (*c* 0·85; MeOH).

Flavonoids. The presence of kampferol-3-glucoside, galangin, and naringenin was confirmed by m.p. with authentic specimens and MS.

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