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ARENOPHTHALIDE A: A NEW PHTHALIDE GLYCOSIDE FROM *HELICHRYSUM ARENARIUM* ROOTS

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Key Word Index—*Helichrysum arenarium*; Compositae; arenophthalide A; phthalide xyloside; structural determination.

Abstract—Spectral data and degradations were used to determine the structure of arenophthalide A, a glycoside of the formula $C_{19}H_{24}O_9$, isolated from the roots of *Helichrysum arenarium*. Structure I was assigned to the compound.

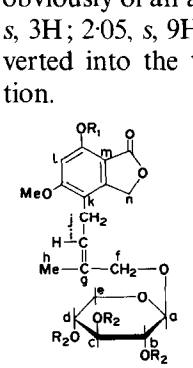
INTRODUCTION

In an early paper [1] on compounds isolated from roots of *Helichrysum arenarium* L. (Moench) the isolation of two glycosides with antibacterial properties was reported. In the present paper, the structure of one of these substances named arenophthalide A has been determined.

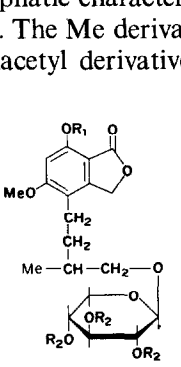
RESULTS AND DISCUSSION

Arenophthalide A (**1**), was a crystalline compound $C_{19}H_{24}O_9$ which shared IR absorption bands at 1520, 1592, 1696, 1730, 3235, and 3380 cm^{-1} and UV maxima at 227, 259 and 296 nm. The MS exhibited only the aglycone peaks, *m/e* 264 ($C_{14}H_{16}O_5$), 246, 231 base peak, and 181 ($C_9H_9O_4$, phthalide nucleus + 2H). The PMR spectrum (for complete PMR data of this substance and all the corresponding derivatives see Table 1) indicated the presence of a Me group on a double bond placed (as shown by double resonance experiments) in an 8 proton $-CH_2-CH=C(Me)-CH_2-$ isoprene fragment (δ 1·69, *bs*, 3H; about 3·25, *d*, 2H; 4·22, *bs*, 2H; 5·29, *bt*, 1H), a methoxy group on an aromatic nucleus (δ 3·78, *s*, 3H), one aromatic hydrogen (δ 6·46, *s*, 1H), six interacting –CH–O– protons, probably of the sugar moiety (δ 2·90–3·60, *m*, 4H; 3·77, *dd*, 1H; 4·10, *d*, 1H), two equivalent isolated –CH–O– protons ascribed to the methylene group in the $Ar-CH_2-O-$ fragment (δ 5·06, *s*, 2H), and finally, four exchangeable OH protons (δ 2·90–3·60, 3H; 4·82, *bs*, 1H).

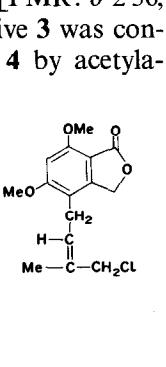
The presence of one phenolic OH group was established by the preparation of the methyl derivative **3**, $C_{20}H_{26}O_9$ [PMR: δ 3·95, *s*, 6H (2 × OMe)] and of the acetyl derivative **2**, $C_{27}H_{32}O_{13}$ containing one acetyl group attached to an aromatic nucleus and three additional acetyl groups, obviously of an aliphatic character [PMR: δ 2·36, *s*, 3H; 2·05, *s*, 9H]. The Me derivative **3** was converted into the triacetyl derivative **4** by acetylation.



(1) $R_1 = R_2 = H$
(2) $R_1 = R_2 = Ac$
(3) $R_1 = Me$; $R_2 = H$
(4) $R_1 = Me$; $R_2 = Ac$



(5) $R_1 = R_2 = H$
(6) $R_1 = R_2 = Ac$
(7) $R_1 = Me$; $R_2 = H$



(8)

The presence of one double bond capable of being hydrogenated, was shown by the preparation of the dihydro derivative **5**, $C_{19}H_{26}O_9$, the PMR spectrum of which changed as expected in the region of the isoprene fragment signals. Acetylation of this dihydro derivative afforded a tetraacetate **6** while methylation led to a Me derivative **7** exhibiting (as the single compound from

Table 1. Characteristic parameters of proton magnetic resonance spectra of arenophthalide A and its derivatives

Compound	Solvent*	Chemical shifts (ppm) H_a	H_b	H_c	H_d	H_e	H_f	H_h	H_i	H_j	H_k	H_n	Other protons
1	A	4·16		2·90 –3·60		3·77	4·22	1·69	5·29	2·90 –3·60	6·46	5·06	3·78 (OMe)
2	B	4·54	4·94	5·21	4·96	3·39 4·15	4·13	1·67	5·30	3·24	6·68	5·16	2·05 (3 × OAc), 2·36 (OAc)
3	A	4·28		3·15 –3·75		3·88	4·21 4·35	1·76	5·32	3·15 –3·73	6·52	5·15	3·95 (2 × OMe)
4	B	4·53	4·95	5·21	4·97	3·38 4·16	4·23 4·39	1·72	5·35	3·26	6·45	5·11	2·05 (3 × OAc), 3·95 (OMe) 3·99 (OMe)
5	A	4·10		2·95 –3·80				0·91	1·15 –1·85	2·42	6·46	5·10	1·15–1·85 (H_g) 3·79 (OMe)
6	B	4·45	4·93	5·19	4·95	3·34 4·11	3·26 3·71	0·96	1·25 1·85	2·44	6·66	5·21	1·25–1·85 (H_g), 2·01 2·03, 2·04, 2·37 (4 × OAc) 3·95 (OMe)
7	A	4·14		2·95 –3·85				0·96	1·20 1·95	2·48	6·55	5·18	1·20–1·95 (H_g), 3·95 (OMe) 3·96 (OMe)
8	A						4·28	1·70	5·30	3·23	6·60	5·15	3·89 (OMe), 3·91 (OMe)
9	B						3·68	1·60	1·93	2·66	6·43	5·16	3·95 (OMe), 3·99 (OMe)
10	A						3·36	0·93	1·10 –1·85	2·48	6·41	5·14	1·10–1·85 (H_g) 3·96 (OMe)
11	A						3·34	0·93	1·10 1·80	2·49	6·53	5·17	1·10–1·80 (H_g), 3·95 (OMe) 3·97 (OMe)
12	B	4·20		2·85 –4·05				1·03	1·25 1·80	2·64	6·41	4·44	3·46, 3·56, 3·60, 3·29 3·82, 3·86 (6 × OMe) 3·82 (COOMe), 1·25–1·85 (H_g)
13	B						3·54	0·99	1·25 –1·90	2·59	6·42	4·45	1·25–1·90 (H_g), 3·35 (OMe) 3·80 (2 × OMe), 6·51 (H_m)
14	B						3·53	0·98	1·15 –1·80	2·63	6·44	4·46	1·15–1·80 (H_g), 3·48, 3·85 3·88 (3 × OMe), 3·83 (COOMe)
15	B	6·88	5·36	5·49	5·26	4·00 4·36							2·05, 2·08, 2·09, 2·11 (4 × OAc), 2·07 (2 × OAc)
16	B	6·28	5·04	5·48	5·03	3·70 3·95							2·02, 2·27 (2 × OAc) 2·05 (2 × OAc)
17	B	5·73	5·03	5·22	4·97	3·52 4·16							2·05 (2 × OAc), 2·10 (OAc)

Compound	Coupling constants (Hz) J_{ab}	J_{bc}	J_{cd}	J_{de}	J_{ee}	J_{ff}	J_{fg}	J_{gh}	J_{hi}	J_{ij}
1–4	6·5	8·0–8·5	8·5	4·5–5·0 8·5–9·0	10·5–12·0	11·5			1·2	7·0–7·5
5–7	6·5	8·5	8·5	5·0; 9·0	12·0	11·5	5·5; 6·5	6·5	0·0	7·0–7·5
8						†			1·2	7·5
9						†			0·0	7·5
10–11, 13–14						†	5·5–6·0	6·5	0·0	7·5–8·0
12	6·5	8·5	8·5	5·0; 9·0	11·5	11·5	5·5; 6·5	6·5	0·0	7·5
15	4·9	4·8	5·8	4·2; 5·9	12·3					
16	3·4	9·5	9·3	5·7; 11·0	10·8					
17	6·4	7·5	8·0	4·6; 8·3	12·0					

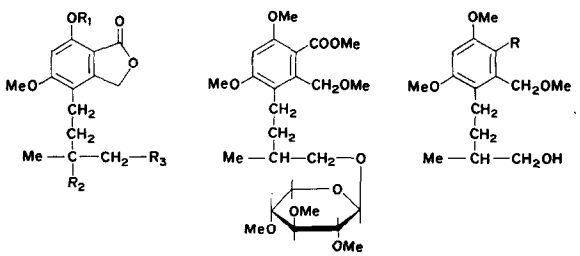
* A = d_6DMSO ≠ $CDCl_3$ (1:1); HMDS as internal reference; B = $CDCl_3$; TMS as internal reference.
† Nonobservable value — both H_f are equivalent.

the arenophthalide A derivatives prepared) an M^+ 412 ($C_{20}H_{28}O_9$), 309 ($C_{16}H_{21}O_6$), 280 ($C_{15}H_{20}O_5$, aglycone), 207 ($C_{11}H_{11}O_4$, benzylic fission), 193 (phthalide nucleus), 179 (207–CO).

Additional structural information was obtained from the hydrolysis of arenophthalide A and its derivatives. The acidic hydrolysis of non-hydrogenated derivatives is very slow; under vigorous conditions, a mixture of about six products is obtained, corresponding to the modified aglycone derivatives which are difficult to separate. On the other hand, alkaline hydrolysis proceeds readily and hydrolysis of the methyl derivative **3** with aqueous alkali affords compounds $C_{15}H_{17}O_4Cl$ and $C_{15}H_{19}O_5Cl$ to which the structures **8** and **9**, respectively, were assigned on the basis of PMR, IR, and MS. The different course of the acidic and alkaline hydrolysis is in accordance with the presence of the allylic glycosidic bond in the molecule. The acidic hydrolysis of arenophthalide A dihydro derivatives proceeds more readily with the formation of a homogenous product. Thus, the hydrolysis of the dihydro derivative **5** afforded a hydroxy derivative **10** which was converted into compound **11** by reaction with diazomethane. The sugar moiety was not isolated in any experiment with either acid or alkaline hydrolysis. On the basis of structural data from

spectra of substituted derivatives **1–7** and hydrolysis products **8–11**, the following partial formula of arenophthalide A may be proposed (R^1–R^4 = H, OH, OMe, CH_2–CH=C(Me)–CH_2O–pentose).

The mutual position of substituents on the aromatic nucleus of arenophthalide A was obtained from the following data. The position of the aromatic proton with respect to the O-containing substituents (OH, OMe) was assigned on the basis of an NOE experiment in PMR of the methylated triacetate **4**. Saturation of the aromatic H signal at δ 6·45 was accompanied by increased relative intensities of the two methoxy group signals at δ 3·95 and δ 3·99 (by 7%). The two methoxy groups must therefore be located in an *ortho* position with respect to the aromatic hydrogen i.e. at positions 4 and 6 or 5 and 7. The distinction between these two alternatives was made by means of the decarboxylated aglycon **13** which was prepared (along with the ester **14**) by permethylation of the dihydro derivative **5** with methyl iodide and sodium hydride in dimethylformamide and subsequent acidic hydrolysis. Thus, in the PMR spectrum of the dihydro derivative **13**, the aromatic H occurring after decarboxylation at the position of the original carbonyl carbon atom, is *meta* with respect to the aromatic H between the two methoxy groups, giving a mutual coupling constant (J 2·5 Hz). The methoxy groups are consequently placed at positions 5 and 7, the aromatic hydrogen at position 6 and the isoprene fragment at position 4.



(9) R_1 = Me; R_2 = OH; R_3 = Cl
(10) R_1 = H; R_2 = H; R_3 = OH
(11) R_1 = Me; R_2 = H; R_3 = OH

(12)

(13) R = H
(14) R = COOMe

Since arenophthalide is a monomethoxy compound, the OH group can either be in position 5 or 7 (in the case of position 5 the IR bands should be influenced by the formation of a 5-membered intramolecular system including the carbonyl group). IR bands ν (C=O) 1730 cm^{-1} and ν (OH) 3235 and 3380 cm^{-1} were observed in the spectrum of the underivatised compound while in the dimethyl derivative **3** these bands were located at 1750 cm^{-1} and 3400 cm^{-1} respectively. The phenolic OH group of arenophthalide A was therefore assigned to position 5 and the methoxy group to position 7 of the phthalide skeleton, as in the simple 7-hydroxy-5-methoxyphthalide isolated from blossoms of the same plant [2].

The structure of the $-CH_2$–CH=C(Me)–CH_2– fragment, attached by means of the first carbon atom to position 4 of the phthalide system and by means of the last carbon atom to the O atom of the pentose (as shown by differences in chemical shifts of the two methylene groups) was established by double-resonance experiments with derivatives **2–5**, and confirmed by PMR of the hydrogenated derivatives **5–7** and **12**, and aglycones **8–11** (Table 1).

The configuration at the double bond of the isoprene fragment was obtained from a NOE experiment on derivative **4**. Saturation of the methyl group signal at δ 1·72 resulted in a significant increase (by 19%) of the relative intensity of the olefinic hydrogen multiplet at δ 5·25, while the signal intensity of the two methylene groups (doublet at δ 3·26 or AB-quartet at δ 4·28) did not change. The configuration of the methyl group and the olefinic H atom on the double bond is therefore *cis*.

The sugar moiety of arenophthalide A was isolated by acetolysis with acetic anhydride and perchloric acid. The reaction product was obtained in the form of the hexaacetate **15**, the saponification of which and the subsequent acetylation yielded α-D-xylopyranose tetraacetate (**16**). The structure of the two substances was confirmed by comparison of their PMR spectra with those of compounds obtained by acetylation of xylose i.e. α-D-xylose tetraacetate (**16**), β-D-xylose tetraacetate (**17**), and hexaacetylxylose (**15**). It was inferred from the chemical shifts and coupling constants of the anomeric H atom in arenophthalide A acetates (**2**, **4**, and **6**; about δ 4·50, $J_{a,b}$ = 6·5 Hz) and those of authentic β-D-xylopyranose tetraacetate (δ 5·73, $J_{a,b}$ = 6·4 Hz) and α-D-xylopyranose tetraacetate (δ 6·28, $J_{a,b}$ = 3·4 Hz), that xylose is bound in arenophthalide by a 1-β-D-glycosidic bond.

The structure of arenophthalide A may thus be unambiguously represented by formula **1**.

EXPERIMENTAL

Mps were measured on a Kofler block. TLC was performed on Si gel G. Optical rotations were measured in MeOH, UV spectra in MeOH, IR spectra in KBr micropellets, MS on a high resolution instrument at 70 eV, and the PMR spectra on a 100 MHz apparatus.

Arenophthalide A **1**. Isolated by the procedure reported earlier [1], mp 184°, $[\alpha]_D^{25}$ −4° (c 0·92, MeOH), IR: ν_{max} 1520, 1592, 1696, 1730, 3235, 3380 cm^{-1}; UV: λ_{max} (log ε) 227 (4·22), 259 (4·02), and 296 (3·75) nm; MS: M^+ not observed, *m/e* 264, ($C_{14}H_{16}O_5$), 246, 231 (base), 181 ($C_9H_9O_4$, phthalide nucleus + 2H). Tetraacetate **2**. C_5H_5N–Ac_2O, mp 142–144° (EtOH), IR ν_{max} 1215, 1492, 1592, 1750 cm^{-1}; MS: M^+ not observed, *m/e* 306, 288 ($C_{16}H_{16}O_5$), 246 ($C_{14}H_{14}O_4$), 231, and fragments of the sugar moiety 259, 199, 157, 139, 97. Monomethyl ether **3**. (CH_2N_2, mp 113° (EtOH), IR: ν_{max} 1492, 1600, 1613, 1752, 3400 cm^{-1}; MS: M^+ not observed, *m/e* 278 ($C_{15}H_{18}O_5$), 260, 245, 207 ($C_{11}H_{11}O_4$, benzylic fission), 195 (phthalide nucleus + 2H). Monomethyl ether triacetate **4**, mp 207–210° (C_6H_6). Dihydro derivative **5** (PtO_2 in MeOH), mp 127–130°, IR: ν_{max} 1520, 1595, 1698, 1730, 3235, 3380 cm^{-1}; MS: M^+ not observed, *m/e* 193 ($C_{10}H_9O_4$), 165 ($C_9H_9O_3$), 176 ($C_{10}H_8O_3$). Dihydro derivative tetraacetate **6**. C_5H_5N–Ac_2O, mp 129–133° (EtOH). Dihydro derivative methyl ether **7**. (CH_2N_2), mp 124–126° (EtOH); IR: ν_{max} 1498, 1612, 1738, 3420 cm^{-1}; MS: M^+ 412 ($C_{20}H_{28}O_9$), *m/e* 309 ($C_{16}H_{21}O_6$), 280 ($C_{15}H_{20}O_5$, aglycone), 207 ($C_{11}H_{11}O_4$, benzylic fission), 193 (phthalide nucleus), 179.

Alkaline hydrolysis. The monomethyl ether **3** (0·1 g) was refluxed for 5 min in EtOH (15 ml) and 2M NaOH (5 ml), the reaction mixture kept at 20° for 18 hr, diluted with H_2O (20 ml), treated with 2M HCl (5 ml), and concentrated under red pres. Preparative TLC in $CHCl_3$ afforded compound **8**, mp 177–183° (EtOH); IR: ν_{max} 1500, 1604, 1620, 1750; MS: M^+ 296 ($C_{15}H_{17}O_4Cl$), 261 ($C_{15}H_{17}O_4$), 247 ($C_{14}H_{15}O_4$), 219 ($C_{12}H_{11}O_4$), 207 ($C_{11}H_{11}O_4$), 194 ($C_{10}H_{10}O_4$), 179 ($C_{10}H_{11}O_3$), together with compound **9**, mp 133–136° (EtOH); MS: M^+ 314 ($C_{15}H_{19}O_5Cl$), 278 ($C_{15}H_{18}O_5$), 220 ($C_{12}H_{12}O_4$), 207 ($C_{11}H_{11}O_4$), 193, 190 ($C_{11}H_{10}O_3$), 179 ($C_{10}H_{11}O_3$).

Acidic hydrolysis of the dihydro derivative **5**. A mixture of the dihydro derivative **5** (100 mg), EtOH (20 ml), and 2N H_2SO_4 (4 ml) was refluxed for 12 hr, cooled and diluted with H_2O. Removal of the solid material afforded compound **10**, mp 210–213° (EtOH); MS: M^+ 266 ($C_{14}H_{18}O_5$), 193 ($C_{10}H_9O_4$, benzylic fission), 176 ($C_{10}H_8O_3$), 165 ($C_9H_9O_3$), which was converted into compound **11** by methylation with CH_2N_2, mp 147–148° (EtOH): IR: ν_{max} 1505, 1620, 1747 cm^{-1}; MS: M^+ 280 ($C_{15}H_{20}O_5$), 207 ($C_{11}H_{11}O_4$, benzylic fission), 193 ($C_{10}H_9O_4$, phthalide nucleus), 190 ($C_{11}H_{10}O_3$), 179 ($C_{10}H_{11}O_3$).

Permethylation of arenophthalide A. NaH (100 mg) was added to a soln of arenophthalide *A* (200 mg) in DMF (10 ml), the mixture stirred for 1 hr and treated with MeI (2 ml). The product was isolated by dilution with H_2O and extraction with $CHCl_3$. TLC in C_6H_6–EtOAc (19:1) gave the amorphous permethyl derivative **12**, MS: M^+ 310 ($C_{17}H_{26}O_5$), 278 ($C_{16}H_{22}O_4$), 263 ($C_{15}H_{19}O_4$), 253 ($C_{13}H_{17}O_5$), 223 ($C_{12}H_{15}O_4$). This derivative (75 mg) was heated at 100° in dioxane (10 ml) and 2M HCl (3 ml). Preparative TLC using C_6H_6–EtOAc (7:3) afforded the amorphous compound **13**. MS: M^+ 268 ($C_{15}H_{24}O_4$), 236 ($C_{14}H_{20}O_3$), 245 ($C_{13}H_{17}O_2$), 195 ($C_{11}H_{15}O_3$), 165, and the amorphous compound **14**, MS: 326 ($C_{17}H_{26}O_6$), 294, 263, 253 ($C_{13}H_{17}O_7$), 239 ($C_{12}H_{15}O_5$), 223 ($C_{12}H_{15}O_5$).

Acetolysis of arenophthalide A. Arenophthalide *A* (200 mg) was added to a 99:1 mixture (5 ml) of Ac_2O and $HClO_4$, the mixture kept at 20° for 10 min, poured onto ice, and extracted with Et_2O to afford hexaacetylxylose (**15**). Saponification with H_2SO_4 in EtOH and subsequent acetylation with Ac_2O in C_5H_5N yielded α-D-xylopyranose tetraacetate (**16**).

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