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MONOHYDROXY- AND 2,5-DIHYDROXY TEREPHTHALIC ACIDS, TWO UNUSUAL PHENOLICS ISOLATED FROM *CENTAURIUM ERYTHRAEA* AND IDENTIFIED IN OTHER GENTIANACEAE MEMBERS

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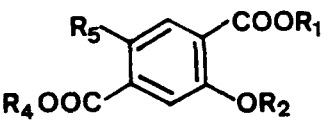
Centaurium erythraea Rafn. (Gentianaceae) has been the subject of previous phytochemical analysis identifying terpenoids (iridoids and secoiridoids) (1,2), polyphenols as flavonols (3) and their *p*-coumaroyl-di- and and tri-*O*-glycosides (4), xanthones characterized by a high oxygenation level (5,6), and, finally, phenolic acids belonging to the benzoic, cinnamic, and phenylacetic groups (4,7). We report here the findings of an examination of a sample of aerial parts from which the terephthalic acids **1** and **2** were isolated; these compounds have been also identified in the MeOH extract of the roots of this species and in other gentianaceous plants.

Analysis of the aqueous infusion and that of the MeOH extract of the aerial parts of *C. erythraea* revealed the presence of two strongly fluorescent products soluble in 2% aqueous $NaHCO_3$. The isolation of these compounds was accomplished by a partitioning of the MeOH extract (dissolved in boiling H_2O) against Et_2O/HCl, followed by a filtration through a Sephadex LH-20 column. Accurate mass measurements for the two compounds **1** and **2** revealed molecular ions corresponding to $C_8H_6O_5$ and $C_8H_6O_6$, respectively. Their identity was established from full analysis of their ^{1}H- and ^{13}C-nmr data and similar data of the permethylated derivatives.

In compound **1**, the 1,2,4-trisubstituted aromatic ring was deduced from the ^{1}H-nmr spectrum (DMSO-d_6) exhibiting three protons at δ 7.86 (d, J = 7.6 Hz, H-6), δ 7.41 (dd, J = 7.6, 1.6 Hz, H-5), and δ 7.39 (d, J = 1.6 Hz, H-3). The low-field region of the ^{13}C-nmr spectrum showed three signals corresponding to two carboxyl functions, δ 171.30 (C-7) and 166.59 (C-8), and to an ethylenic *O*-bound carbon at δ 160.77 (C-2). The absence of any bathochromic uv shift with NaOMe indicated an hydroxyl group in the ortho position to a carboxyl function (8). This result was in agreement with the different δ values recorded for the two -COOH groups (δ 171.30 and δ 166.59), the deshielded one being due to the one chelated to the phenolic group, as shown in Table 1 for 2-hydroxybenzoic acids. C-1 (δ 117.06) was naturally shielded by the ortho-hydroxylation, contrary to C-4 (δ 136.95), which was not affected by the shielding



1 $R_1=R_2=R_4=R_5=H$
2 $R_1=R_2=R_4=H$, $R_5=OH$
3 $R_1=R_2=R_4=Me$, $R_5=H$
4 $R_1=R_2=R_4=Me$, $R_5=OMe$

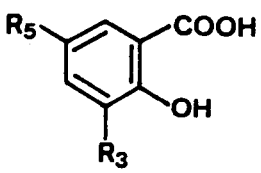
TABLE 1. ^{13}C-nmr Chemical Shifts of Hydroxyterephthalic Acid [**1**], 2,5-Dihydroxyterephthalic Acid [**2**], Salicylic Acid [**5**], 2,3-Dihydroxybenzoic Acid [**6**], and 2,5-Dihydroxybenzoic Acid [**7**] (50 MHz; DMSO-d_6; δ ppm/TMS).

Carbon	Compound				
	1	**2**	**5**	**6**	**7**
C-1	117.06	121.40	113.13	113.45	112.91
C-2	160.77	152.69	161.52	150.73	154.55
C-3	117.86[a]	116.50	117.31[a]	146.18	118.12[a]
C-4	136.95	121.40	135.80	121.06[a]	124.13
C-5	119.62[a]	152.69	119.32[a]	118.88	149.71
C-6	130.80	116.50	130.53	120.36[a]	114.94[a]
C-7	171.30	170.92	172.32	172.83	172.15
C-8	166.59	170.92			

[a]Assignment confirmed by selective irradiation of the corresponding proton.

effect of the involved substituent. Finally, compound **1** was considered hydroxyterephthalic acid, consequent to nOe recorded in C_6D_6 between the isolated H-3 (δ 7.62) and MeO-2 (δ 3.25) of the trimethyl derivative **3**.

With a supplementary oxygen atom belonging to a phenolic function, compound **2** exhibited only four signals in the ^{13}C-nmr spectrum (δ 170.92, 152.69, 121.40, and 116.50; C-7,8, C-2,5, C-1,4, and C-3,6), and its ^{1}H-nmr spectrum was reduced to one singlet at δ 7.15 (H-3,6). Compound **2** was deduced to be 2,3-dihydroxyterephthalic acid or 2,5-dihydroxyterephthalic acid. The first hypothesis was ruled out on the basis of nOe's recorded between the equivalent H-3,6 (δ 7.40) and the equivalent MeO-2,5 (δ 3.29) of the tetramethyl derivative **4** in C_6D_6. This result was confirmed by the comparative analysis of $\Delta\delta_{C\text{-}2}$ and $\delta\Delta_{C\text{-}6}$ between terephthalic acids **2** and **1** and between 2,5-dihydroxybenzoic acid [**7**] and salicylic acid [**5**] (Table 2). The shielding effect produced by the supplementary 5-OH was more consequent on the ortho carbon (C-6), than on the para carbon (C-2), as indicated by the ratio $\Delta\delta_{C\text{-}6}/\Delta\delta_{C\text{-}2} = 1.77$ for the terephthalic acids and 2.24 for the couple 2,5-dihydroxybenzoic acid-salicylic acid. Inversely, owing to the 3-hydroxylation, this ratio decreased to 0.88 between 2,3-dihydroxybenzoic acid [**6**] and salicylic acid.



5 $R_3=R_5=H$
6 $R_3=OH$, $R_5=H$
7 $R_3=H$, $R_5=OH$

TABLE 2. Comparative Analysis of $\Delta\delta_{C\text{-}2}$ and $\Delta\delta_{C\text{-}6}$ Showing the Shielding Effect Produced by the Supplementary OH group on C-2 and C-6 Between 2,3-Dihydroxy Benzoic Acid [**6**] and Salicylic Acid [**5**], 2,5-Dihydroxy Benzoic Acid [**7**] and Salicylic Acid [**5**], Hydroxyterephthalic Acid [**1**] and 2,5-Dihydroxyterephthalic Acid [**2**].

	δ6-δ5	δ7-δ5	δ2-δ1
$\Delta\delta_{C\text{-}2}$	−10.79	−6.97	−8.08
$\Delta\delta_{C\text{-}6}$	−9.47	−15.59	−14.30
$\Delta\delta_{C\text{-}2}/\Delta\delta_{C\text{-}6}$ . .	0.88	2.24	1.77

Surprisingly, monohydroxyterephthalic acid and 2,5-dihydroxyterephthalic acid do not appear to have been reported previously as natural products. Hydroxyterephthalic acid has been just described as one of the metabolites of forphenicinol [L-(3-hydroxy-4-hydroxymethylphenyl)-

glycine] in animals (rat, rabbit, and dog) but not in man (9). To know if these two natural products are restricted to *C. erythraea* or not, other available gentianaceous plants have been analyzed. The two terephthalic acids have been recovered in the aerial parts of *Blackstonia perfoliata* (L.) Huds., *Gentiana lutea* L., *Gentiana punctata* L., *Gentiana purpurea* L., *Swertia chirata* Buch. Ham., *Swertia perennis* L., and in the roots of *G. purpurea* and *S. chirata*. They have not been detected in the aerial parts of *Menyanthes trifoliata* L. and the seeds of *G. lutea*. It will be interesting to know if these phenolic acids are widespread in the vegetal kingdom or if they are specific to the Gentianaceae members.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURE.—Analytical tlc was carried out on Si gel plates 60F-254 (E. Merck) and microcrystalline cellulose Polygram cel 400 UV254 (Macherey Nagel). Sephadex LH20 (Pharmacia Fine Chemicals) was used for cc. Uv spectra were measured in MeOH on a Beckman 25 spectrometer. ^{1}H- and ^{13}C-nmr spectra (DMSO-d_6; C_6D_6; δ ppm/TMS) were recorded on an AC200 Bruker nmr spectrometer. Ir spectra (KBr) were taken on an Unicam SP1100 spectrometer. Ei mass spectra were obtained with an AEI MS902 mass spectrometer (70 eV).

PLANT MATERIAL.—*C. erythraea* was previously reported (5); *G. lutea* (GL-75) was collected in July 1975, at Col de la Charmette, Isère, France; *G. punctata* (GP-75) was collected in August 1975, at Col d'Arsine, Hautes Alpes, France; *G. purpurea* (GPu-75) was collected in August 1975, at Col du Petit Saint Bernard, Savoie, France; *S. perennis* (SwP-75) was collected in July 1975, at Col du Lautaret, Hautes Alpes, France; *S. chirata* (SwC-76) was a generous gift from la Mission CNRS-Népal-RCP 253; *B. perfoliata* (BP-72) was a gift from Prof. P. Lebreton, Lyon; *M. trifoliata* (MT-85) was purchased from Gifrer & Barbezat, Lyon. All the voucher specimens are deposited at Laboratoire de Pharmacognosie de Grenoble, Domaine de la Merci, F-38700 La Tronche.

EXTRACTION AND IDENTIFICATION OF TEREPHTHALIC ACIDS **1** AND **2**.—Each of the above-mentioned species (10 g), first extracted with *n*-hexane and then with $CHCl_3$ at room temperature, was treated with MeOH. After concentration, the MeOH extract was dissolved in H_2O, acidified to pH 2 with 2 N HCl, and then partitioned against Et_2O. The upper phase was extracted by aqueous 2% $NaHCO_3$ which was acidified to pH 2 with 4 N HCl before being partitioned against Et_2O. After concentration, the organic layer was used for tlc analysis in the systems cellulose *n*-BuOH-HOAc-H_2O (4:1:6, upper phase), cellulose HCOONa-HCOOH-H_2O (5:0.5:100), and Si gel C_6H_6-HOAc-H_2O (60:22:1.2) in the presence of authentic samples of hydroxy- and 2,5-dihydroxyterephthalic acids isolated from *C. erythraea* aerial parts.

ISOLATION OF THE TEREPHTHALIC ACIDS **1** AND **2**.—*C. erythraea* roots (1 kg) were extracted by petroleum ether (20 liters) at room temperature and then by boiling MeOH (20 liters). After concentration under vacuo, the MeOH extract was dissolved in boiling H_2O and subjected to filtration. The soluble part was extracted successively by Et_2O (15 liters) and *n*-BuOH (15 liters). Terephthalic acids **1** and **2** were found in the Et_2O extract (7 g). A part of this extract (1 g) was submitted to Sephadex LH-20 cc packed with MeOH-iPrOH (60:40); terephthalic acid **2**, exhibiting a strong yellow fluorescence, was first eluted, followed by terephthalic acid **1**, characterized by a strong blue fluorescence. Sixteen fractions were collected and monitored by tlc in the above-mentioned systems. Fractions 4–7 were used for purification of terephthalic acid **2** (12 mg) by Sephadex LH-20 cc (MeOH), and fractions 9–11 were subjected to preparative tlc on cellulose in using the eluting mixture HCOONa-HCOOH-H_2O (5:0.5:100), followed by filtration through a Sephadex LH-20 column (MeOH) to purify terephthalic acid **1** (9 mg).

HYDROXYTEREPHTHALIC ACID [**1**].—Cream-colored, amorphous powder; uv λ MeOH 245, 320; /$AlCl_3$ 255, 262 sh, 337; /$AlCl_3$ + HCl = /MeOH; /NaOMe 242, 310 nm; ir ν KBr 3400, 2860, 2838, 1710, 1690, 1670, 1500, 1432, 1389, 1300, 1240, 1220, 1210, 755 cm^{-1}; eims (70 eV) *m/z* (%) $[M]^+$ 182 (35), (182.0210; $C_8H_6O_5$ = 182.0215, $[M-H_2O]^+$ 164 (100), $[M-H_2O-CO]^+$ 136 (30), $[M-2H_2O-CO+H]^+$ 119 (50); ^{1}H nmr (200 MHz; DMSO-d_6) δ 7.86 (1H, d, J = 7.6 Hz, H-6), 7.41 (1H, dd, J = 7.6, 1.6 Hz, H-5), 7.39 (1H, d, J = 1.6 Hz, H-3); ^{13}C nmr see Table 1.

METHOXY-DIMETHYLTEREPHTHALATE [**3**].—From compound **1**, CH_2N_2/Et_2O, room temperature, 2 h: yellow mass purified by circular centrifugal tlc using the system Si gel C_6H_6-MeOH (94:6); colorless needles obtained after crystallization in MeOH; uv λ MeOH 245, 307 nm; ^{1}H nmr (200 MHz; C_6D_6) δ 7.75 (1H, d, J = 7.6 Hz, H-6), 7.66 (1H, dd, J = 7.6, 1.4 Hz, H-5), 7.62 (1H, d, J = 1.4 Hz, H-3), 3.54 (3H, s, COOMe), 3.49 (3H, s, COOMe), 3.25 (3H, s, MeO-2); ^{13}C nmr (50 MHz; C_6D_6) δ

166.78 (1C, C-7 or C-8), 166.64 (1C, C-7 or C-8), 159.69 (1C, C-2), 135.32 (1C, C-4), 132.24 (1C, C-6), 122.07 (1C, C-5), 113.91 (1C, C-3), 56.12 (1C, MeO-2), 52.49 (1C, COO*Me*), 52.36 (1C, COO*Me*).

2,5-DIHYDROXYTEREPHTHALIC ACID [**2**].—Yellow amorphous powder; uv λ MeOH 252, 372; /$AlCl_3$ 260, 405; /$AlCl_3$ + HCl 254, 376; /NaOMe 250, 352; /NaOAc 250, 350; /NaOAc + H_3BO_3 256, 374 nm; ir ν KBr 3500, 1655, 1500, 1430, 1360, 1290, 1260, 1200, 1100, 900, 850, 784, 755 cm^{-1}; eims (70 eV) *m/z* (%) $[M]^+$ 198 (48), (198.01635, $C_8H_6O_6$ = 198.01640), $[M-H_2O]^+$ 180 (100), $[M-2H_2O]^+$ 162 (97), $[M-2H_2O-CO]^+$ 134 (43), 107 (7), 106 (10); ^1H nmr (200 MHz, DMSO-d_6) δ 7.15 (2H, s, H-3,6); ^{13}C nmr see Table 1.

2,5-DIMETHOXY-DIMETHYLTEREPHTHALATE [**4**].—From compound **2**, CH_2N_2/Et_2O, room temperature, 2 h: yellow mass purified as for **3**; colorless needles; uv λ MeOH 242, 333 nm; ir ν KBr 1715, 1500, 1430, 1400, 1300, 1240, 1222, 1215, 1170, 1120, 1030, 935, 880, 840, 800, 785, 755 cm^{-1}; eims (70 eV) *m/z* (%) $[M]^+$ 254 (100) (254.07908, $C_{12}H_{14}O_6$ = 254.07900), $[M-Me]^+$ 239 (20), $[M-OMe]^+$ 223 (60), 221 (30), $[M-Me-OMe]^+$ 208 (20), 149 (70); ^1H nmr (200 MHz; C_6D_6) δ 7.40 (2H, s, H-3,6), 3.59 (6H, s, 2COOMe), 3.29 (6H, s, MeO-2,5); ^{13}C nmr (50 MHz; C_6D_6) δ 166.88 (2C, C-7,8), 153.34 (2C, C-2,5), 116.52 (2C, C-3,6), 115.87 (2C, C-1,4), 56.71 (2C, MeO-2,5), 52.53 (2C, MeO-7,8).

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