Pergamon

0031-9422(93)E0016-8

Phytochemistry, Vol. 35, No. 5, pp. 1349 1354, 1994

0031 9422/94 $6.00+0.00

TAMARIXELLAGIC ACID, AN ELLAGITANNIN FROM THE GALLS OF *TAMARIX APHYLLA*

M. A. M. NAWWAR, S. A. M. HUSSEIN, J. BUDDRUS* and M. LINSCHEID*

National Research Centre, El-Dokki, Cairo, Egypt; *Institut für Spektrochemie, Postfach 778, W-4600 Dortmund 1, Germany

(*Received* 25 *August* 1993)

Key Word Index—*Tamarix aphylla*; Tamaricaceae; galls; ellagitannins; tamarixellagic acid; dehydrotrigallic acid; NMR.

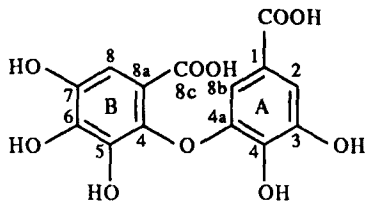
Abstract—The novel natural polyphenol, 2-*O*-galloyl-3-*O*-(3,4,5,6,7-pentahydroxybiphenyl ether-8_a-carboxylic acid-1-carboxyloyl)-4,6-(*S*)-hexahydroxybiphenoyl-(α/β)-$^4C_1$-glucopyranose, tamarixellagic acid, together with the known dehydrodigallic and dehydrotrigallic acids were isolated from the galls of *Tamarix aphylla*. The structures were established by conventional methods of analysis and confirmed by spectral procedures.

INTRODUCTION

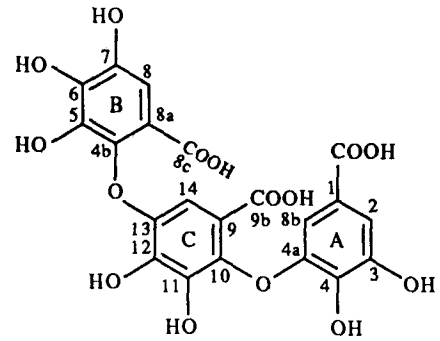
Whilst almost all parts of the Egyptian tamarix plants, *Tamarix nilotica* and *T. aphylla*, have been comprehensively studied for their phenolics, only preliminary phytochemical investigations [1, 2] have been carried out on the galls of the latter. Gallic, dehydrodigallic, isoferulic acids, besides the flavonol glucosides, tamarixin and isoquercitrin, have been characterized by routine methods of analysis. In the present communication, we describe the isolation and structural elucidation of the new ellagitannin, 2-*O*-galloyl-3-*O*-(3,4,5,6,7-pentahydroxybiphenyl ether-8_a-carboxylic acid-1-carboxyloyl)-4,6-(*S*)-hexahydroxybiphenoyl-(α/β)-$^4C_1$-glucopyranose or tamarixel-lagic acid. The rare polyphenols dehydrodigallic and dehydrotrigallic acids were also isolated and identified. The NMR analysis of dehydrodigallic acid xanthone, dehydrotrigallic acid and dehydrotrigallic acid xanthone (see formulae) were recorded and assigned for the first time. It should be noted that dehydrotrigallic acid has been isolated once before, from the hydrolysate of *Myricaria alopecuroides* (Tamaricaceae) extract and was then characterized through elemental, chromatographic and IR analyses, as well as through colour and precipitation reactions, and preparation of the methyl derivative [3].

RESULTS AND DISCUSSION

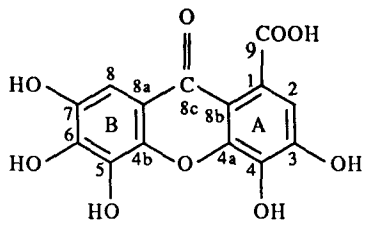
The aqueous ethanolic gall extract of *T. aphylla* was shown by preliminary 2D-PC screening to contain a complicated phenolic mixture from which three compounds (**1**–**3**) were isolated and purified by polyamide CC, followed by Sephadex LH-20 CC and preparative PC. One of the separated compounds (**3**) is new. The remaining compounds (**1** and **2**) are known and gave chromatographic, UV spectral, hydrolytic and NMR data of **1** and **2** identical with those of dehydrodigallic acid and dehydrotrigallic acid, respectively (Table 1) [2–4]. The structure of **1** was then confirmed through dehydration by conc. H_2SO_4 to yield the known xanthone, 3,4,5,6,7-pentahydroxyxanthone-1-carboxylic acid [5] and the subsequent NMR spectral analysis of the crystalline dehydration product (**1a**). The ^1H and ^{13}C NMR spectra are recorded and assigned for the first time. In the ^1H NMR spectrum, two singlets at δ7.04 and 7.06 ppm were recognized and assigned to the aromatic protons H-2 and H-8 (see formula), while the remaining broad singlet resonating at δ8.24 ppm was assignable to the carboxylic proton. The ^{13}C NMR spectrum revealed 14 distinct resonances. For the assignment of these resonances, substituent increment rules have been deduced by comparing the chemical shift values reported for biphenyl ether [6] with those given for unsubstituted xanthone [7]. These rules were then applied to the ^{13}C NMR chemical shifts recorded previously for monodecarboxydehydrodigallic acid [8], a natural pentahydroxybiphenyl ether monocarboxylic acid which possesses the same substitution pattern as that of the investigated xanthone (**1a**). However, unambiguous assignments could be achieved only by measuring the ^1H–^{13}C coupling constants. Among the 14 signals thus recorded, the furthest two low-field resonances at δ174.3 (*d*, $J = 4$ Hz) and 169.7 ppm (*d*, $J = 4$ Hz) were assignable to the C=O carbons (C-8_c and C-9), respectively (see formulae). The signals of the protonated carbons C-2 and C-8 were identified by their one-bond couplings (δ112.9, *d*, $J = 165$ Hz and 99.5 ppm, *d*, $J = 165$ Hz, respectively). Couplings across three bonds were also found for C-4, C-6, C-4_b and C-8_b (all appeared as doublets with $J \simeq 10$ Hz). The resonances of C-1, C-3, C-7 and C-8_a showed coupling across two bonds ($J = 1.5$ or 4 Hz), whereas those of C-4_a and C-5 were split over four bonds ($J = 1$ Hz). The structure of **2** as dehydrotrigal-



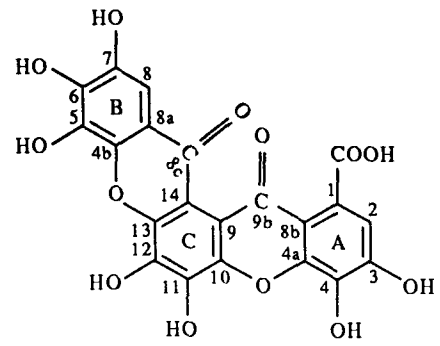
dehydrodigallic acid, (1)



dehydrotrigallic acid, (2)

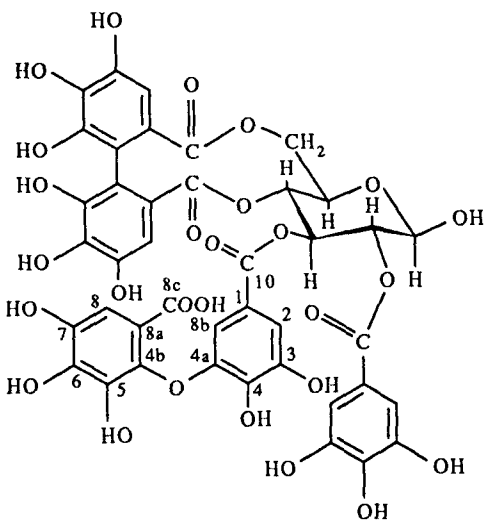


dehydrodigallic-xanthone, (**1a**)



dehydrotrigallic-xanthone, (**2a**)

Nb.: Numbering of the carbon atoms in the above given formulae is for convenience.



3 β-form

lic acid has been proved through negative FAB-MS, whereby a molecular ion peak has been recognized in the received spectrum, at (M − H): 504.8, corresponding to a M_r of 506. Further confirmation of this structure has been obtained through NMR spectral analysis. The spectrum, recorded and assigned for the first time, exhibited the structural resemblance of this structure with that of dehydrodigallic acid, whose configuration was previously determined [4]. The ^{1}H NMR spectrum showed the characteristic dehydrodigallic acid proton pattern of signals (δ7.02, *d*, J = 2.5 Hz; 6.95, *s* and 6.48 ppm, *d*, J = 2.5 Hz), together with a sharp singlet located relatively upfield at δ6.42 ppm and integrated to one proton, assignable to H-14 proton (see formulae). These data prove that **2** is built up from a dehydrodigallic acid moiety coupled to a gallic acid moiety through dehydrogenation and ether bridge formation connecting C-7 of the former to C-2 (or 6) of the latter. The upfield position of the H-14 signal could then be attributed to the anisotropy of ring B which is out of the plane of ring C. An ether bridge joining dehydrodigallic acid to gallic acid, at positions other than those stated above, would produce derivatives which could not lose two molecules of water when dehydrated by conc. H_2SO_4, in contrast with **2** whose dehydration product exhibited, in negative FAB-MS, a molecular ion peak at (M − H): 468.8, corresponding to a M_r of 470, thus proving the loss of two water molecules from **2** to yield a benzo-γ-pyronoxanthone (**2a**). This view was finally confirmed through ^{13}C NMR analysis of **2**. The recorded spectrum revealed the presence of 21 distinct carbon resonances, of which 14 were found to possess chemical shifts typical for the A and B ring carbons of dehydrodigallic acid (Table 2). The remaining seven resonances belonging to the carboxylated ring C of **2** were assigned by applying the substituent rules deduced when comparing the chemical shifts of gallic acid with

Table 1. Chromatographic and UV data of the polyphenols

Compound no.	Chromatographic properties R_fs (×100) H_2O	HOAc-6	BAW	UV Spectral data λ_{max}^{MeOH} (nm)
1	45	58	75	270
1a	15	43	48	364, 310^{inflection}, 260, 245
2	45	54	49	269
2a	10	21	40	430, 320, 265, 242
3	40	53	47	273
gallic acid	53	56	78	272
ellagic acid	00	08	46	362, 255
4,6-HHDP-glucose	55	65	22	267
3a	47	61	36	273

Table 2. ^{13}C chemical shifts (ppm) of the investigated phenolics

C*	a	1	2	x	1a	2a
1	120.6	120.6	119.7	121.7	125.3 (*d*, $J=1.5$)	124.2 (*d*, $J=1.5$)
2	108.8	107.1	105.8	106.8	112.9 (*d*, $J=165$)	114.5 (*d*, $J=165$)
3	145.5	146.1	145.4†	146.5	148.9 (*d*, $J=1.5$)	148.1 (*d*, $J=1.5$)
4	138.1	139.6	138.8†	140.4	139.8 (*d*, $J=7$)	138.6 (*d*, $J=7$)
4a	145.5	148.0	147.2	147.3	145.4 (*d*, $J=1.0$)	145.4 (*d*, $J=1.0$)
8b	108.8	111.3	110.4	111.9	110.8 (*d*, $J=7$)	111.0 (*d*, $J=7$)
10			135.6			136.5 (*s*)
11			139.7			144.8 (*s*)
12			139.9			143.9 (*s*)
13			144.0			137.3 (*s*)
14			108.2			114.8 (*d*, $J=1$)
9			114.2			110.2 (*s*)
4b		136.6	137.2	138.8	133.9 (*d*, $J=7$)	133.7 (*d*, $J=7$)
5		140.0	139.5†	137.4	133.8 (*d*, $J=1.0$)	133.1 (*d*, $J=1$)
6		139.7	139.1†	135.1	140.7 (*d*, $J=7$)	139.2 (*d*, $J=7$)
7		143.0	142.5	143.7	144.0 (*d*, $J=1.5$)	144.1 (*d*, $J=1.5$)
8		109.0	107.0	110.1	99.5 (*d*, $J=165$)	99.3 (*d*, $J=165$)
8a		115.7	114.9	112.3	112.4 (*d*, $J=1.5$)	113.7 (*d*, $J=1.5$)
1b	167.7	168.2	167.3	167.5	169.6 (*d*, $J=4$)	168.9 (*d*, $J=4$)
9b			166.1			172.2 (*s*)
8c		167.1	166.1		174.3 (*d*, $J=4$)	173.3 (*d*, $J=4$)

Compound: (a): gallic acid; (**1**): dehydrodigallic acid; (**2**): dehydrotrigallic acid; (x): 8a-decarboxydehydrodigallic acid, data from ref. [8]; (**1a**): dehydrodigallic xanthone; (**2a**): dehydrotrigallic xanthone.

*Numbering is for convenience.

†Signals could be reversed.

those of dehydrodigallic acid [2-(4,5-dihydroxy-3-oxy-1-carboxylic)phenyl gallic acid] on the chemical shifts of ring B carbons of dehydrodigallic acid itself. This results in calculated chemical shifts for the C ring carbons of dehydrotrigallic acid. Both calculated and measured chemical shifts were in close agreement and led to the assignment of the resonances at δ135.6, 139.7, 139.9 and 144.2 ppm to the oxygenated ring C carbons C-10, C-11, C-12 and C-13, respectively, while those at δ108.2 and 114.2 ppm were assignable to the protonated carbon C-14 and the quaternary carbon C-9. The NMR spectra of the benz-γ-pyronoxanthone (**2a**) were also recorded and assigned for the first time. In the ^{1}H NMR spectrum only two aromatic sharp singlets of equal integration have been recorded at δ7.0 and 6.93 ppm. These were assigned on the basis of their very close chemical shifts to the H-2 and H-8 protons in the spectrum of the parent compound (**2**), and to the same protons in the molecule of the investigated derivative (**2a**). The ^{13}C spectrum of **2a** exhibited 21 resonances, among which 14 were found to possess chemical shifts similar to those measured for the carbon signals of the dehydrodigallic xanthone (Table 2). The remaining seven signals, attributable to the carbons of ring C and the connected C=O group, were assigned by comparison with the calculated shift values obtained by applying the substituent increment rules to the measured chemical shifts of ring B of the dehydrodigallic xanthone (**1a**).

The phenolic **3**, isolated as a light brown amorphous powder of $[\alpha]_D$ +132° (MeOH; *c* 1.3) was found to possess chromatographic properties, colour reactions (dark blue with $FeCl_3$ and violet with nitrous acid spray reagents on PC) and UV spectral data consistent with an ellagitannin. It exhibited a molecular ion peak at $[MH]^+$ 955 in positive FAB-MS and at (M−H) 953 in negative FAB-MS, corresponding to a M_r of 954. On complete acid hydrolysis, **3** yielded glucose (co-PC), gallic, ellagic and dehydrodigallic acids (co-PC). The released ellagic acid, precipitated from the cold aqueous hydrolysate was fully characterized through UV, ^1H and ^{13}C NMR spectral analysis, while the released gallic and dehydrodigallic acids were individually separated by polyamide CC of their ethyl acetate extract, using H_2O–EtOH mixtures of decreasing polarities for elution. The identity of each was then confirmed by UV, ^1H and ^{13}C NMR spectral analysis. On controlled acid hydrolysis, **3** yielded, among other products, 4,6-*O*-hexahydroxybiphenoylglucose (co-PC, UV spectral data and positive FAB-MS) together with an ellagitannin intermediate (**3a**) which appeared on 2D-PC as a dark blue spot in UV light turning dark blue when sprayed with $FeCl_3$ and violet on spraying with nitrous acid [9]. A pure amorphous sample of **3a**, obtained through preparative PC of the ethyl acetate extract of the controlled acid hydrolysis products, was found to possess $[\alpha]_D$ +70° (MeOH; *c* 0.5) and UV spectral data similar to that of **3**. Positive and negative FAB-MS of **3a** showed the molecular ion peaks, $[MH]^+$ 803 and (M−H) 801, respectively, thus proving a M_r of 802. Hence, **3a** is formed through the mono-esterification of a 4,6-*O*-hexahydroxybiphenoylglucose moiety with a dehydrodigallic acid moiety. This assumption was then proved through complete acid hydrolysis of **3a** to yield glucose, ellagic and dehydrodigallic acids (co-PC). Consequently, **3** is the monogalloyl derivative of **3a**. To find out the site of attachment of the galloyl and dehydrodigalloyl moiety to the 4,6-hexahydroxybiphenoylglucose moiety to form **3**. ^1H NMR spectral analysis was then engaged. The spectrum revealed two distinct patterns of proton signals belonging to substituted α- and β-glucose anomers. Each pattern was found to contain well separated signals of the seven-spin system belonging to a distinct glucose anomer. The spectrum also showed one pair of singlets in the aromatic region for the galloyl moieties (one for each anomer), as well as two pairs of singlets for the hexahydroxybiphenoyl protons (one pair for one moiety in each anomer). The characteristic pattern of dehydrodigallic acid proton signals has revealed itself twice in this spectrum. The appearance of two signals for each distinct proton in **3** proved the presence of a free anomeric glucose hydroxyl group, which restricts the site of attachments of the galloyl and dehydrodigalloyl moieties to the glucose positions 2 and 3. The ambiguity in determining the site of attachments between the galloyl, dehydrodigalloyl and the 4,6-hexahydroxybiphenoyl glucose moieties to form **3** was then unravelled through measurement of ^1H NMR spectrum of the intermediate **3a** and the subsequent comparison between the spectrum and that of **3**. This comparison has shown the disappearance of the galloyl proton signals from the spectrum of **3a** which was accompanied by a large upfield shift of the H-2-α and H-2-β glucose proton resonances on comparison with the positions of both signals in the spectrum of **3** (Table 3). The recognition that the remaining sugar and phenolic proton signals in the spectrum of **3a** have almost the same chemical shift values and multiplicities as those of the corresponding signals in **3**, confirmed that the galloyl moiety which was released from the parent compound during controlled acid hydrolysis to produce the intermediate **3a** was esterifying the glucose at C-2, leaving the OH at C-3 to be esterified by one of the carboxylic groups of the dehydrodigalloyl moiety, as was concluded from the downfield positions of the geminal H-3-α and H-3-β-glucose proton signals in the spectra of both **3** and **3a** (in comparison with the corresponding signals in the spectrum of α- and β-glucose [10]). Thus, **3** is a (*S*)-4,6-hexahydroxybiphenoyl-(α/β)-glucose which is esterified

Table 3. ^1H chemical shifts (ppm) and coupling constants (Hz) of the investigated phenolics

	Protons of the glucose moiety						
Compound	H-1	H-2	H-3	H-4	H-5	H-6	H-6′
(**3**)-α	5.28 (*d*, *J* = 2.5)	4.88 (*m*)	5.68 (*t*, *J* = 8)	4.88 (*m*)	4.5 (*m*)	5.16 (*m*)	3.78 (*d*, *J* = 12)
(**3**)-β	4.84 (*d*, *J* = 8)	4.98 (*m*)	5.42 (*t*, *J* = 8)	4.88 (*m*)	4.15 (*m*)	5.16 (*m*)	3.72 (*d*, *J* = 12)
(**3a**)-α	5.22 (*d*, *J* = 2.5)	3.75 (*m*)	5.61 (*t*, *J* = 8)	4.85 (*m*)	4.45 (*m*)	5.07 (*m*)	3.75 (*m*)
(**3a**)-β	4.45 (*d*, *J* = 8)	3.75 (*m*)	5.36 (*t*, *J* = 8)	4.85 (*m*)	4.48 (*m*)	5.07 (*m*)	3.75 (*m*)
	Aromatic galloyl protons		Aromatic dehydrodigallicmonocarboxyloyl			Aromatic hexahydroxydiphenoyl protons	
(**3**)-α/β	6.75 (*s*), 6.83 (*s*)		6.42 (*d*, *J* = 2.5); 6.46 (*d*, *J* = 2.5); 6.82 (*s*); 6.88 (*s*); 7.0 (*d*, *J* = 2.5); 7.08 (*d*, *J* = 2.5)			6.2 (*s*), 6.23 (*s*), 6.33 (*s*), 6.34 (*s*)	
(**3a**)-α/β			6.39 (*d*, *J* = 2.5); 6.42 (*d*, *J* = 2.5); 6.84 (*s*); 6.88 (*s*); 6.95 (*d*, *J* = 2.5); 7.0 (*d*, *J* = 2.5)			6.28 (*s*), 6.3 (*s*), 6.32 (*s*), 6.35 (*s*)	

Compound **3**: 2-mono-*O*-galloyl-3-mono-*O*-dehydrodigallicmonocarboxyloyl 4,6-(*S*)hexahydroxydiphenoyl-(α/β)-$^4C_1$-glucopyranose; compound **3a**: 3-*O*-mono-*O*-dehydrodigallicmonocarboxyloyl 4,6-(*S*)hexahydroxydiphenoyl-(α/β)-$^4C_1$-glucopyranose.

at positions 2 and 3 of its glucose core by galloyl and dehydrodigalloyl moieties, respectively.

The ^{13}C NMR analysis of **3** has confirmed this structure. As expected, the spectrum exhibited double signals for each carbon. The α- and β-anomers were recognized from the downfield anomeric glucose carbon resonances at δ89.5 and 95.3 ppm, respectively, while the most upfield signal at δ62.5 was assigned to the C-6 glucose carbon in both anomers. Assignments of the remaining glucose carbon signals were aided by comparison with the recorded chemical shifts of 2,3-di-*O*-galloyl-(α/β)-$^4C_1$-glucopyranose [11], as well as with those reported for galloylated 4,6-*O*-hexahydroxybiphenoylglucoses [9, 12]. Presence of only one galloyl moiety in **3** followed from the two galloyl C=O carbon resonances (one for each anomer) at δ165.4 and 165.3 ppm, while the presence of a dehydrodigalloyl moiety was apparent from the appearance of the typical dehydrodigallic acid pattern of carbon signals (twice). However, one of the carboxyl carbons of the latter moiety has revealed its signal twice at δ162.6 and 162.4 ppm, a location which is upfield when compared with that of the free dehydrodigallic acid carboxyl carbon signals (Table 2). This shift is obviously due to the esterification of this carboxyl group with the alcoholic glucose OH group at position 3. Both of these upfield carboxyl carbon signals have appeared as a triplet of J = 4.5 Hz in the ^{1}H–^{13}C coupled spectrum of **3**, thus confirming that they belong to the carbon of the COOH group 10 (see formulae), which is coupled to the H-2 and H-8$_b$ protons to result in the detected triplet signals (one for each anomer). Furthermore, the measured chemical shift values of the sugar carbon signals confirmed that the sugar core exists in the pyranose form. Consequently, **3** is 2-*O*-galloyl-3-*O*-(3,4,5,6,7-pentahydroxybiphenyl ether-8$_a$-carboxylic acid-1-carboxyloyl)-(*S*)-4,6-hexahydroxybiphenoyl-(α/β)-$^4C_1$-glucopyranose, which has not been reported before in the literature.

EXPERIMENTAL

^{1}H NMR chemical shifts were measured relative to TMS and ^{13}C NMR chemical shifts relative to DMSO-d_6, and converted to the TMS scale by adding 39.5. Typical conditions: spectral width = 6000 Hz for ^{1}H and 22 000 Hz for ^{13}C, 32 K data points and a flip angle of 45°. ^{1}H–^{13}C coupled NMR spectra were obtained by the gated decoupling technique. PC was carried on Whatman No. 1 paper, using solvent systems: (i) H_2O; (ii) HOAc (HOAc–H_2O, 3:17); (iii) BAW (*n*-BuOH–HOAc–H_2O, 4:1:5, top layer); (iv) C_6H_6–*n*-BuOH–pyridine–H_2O (1:5:3:3, top layer). Solvent system iii was used for prep. PC on Whatman No. 3MM paper, while solvent systems iii and iv were used for sugar analysis.

Plant material. Fresh galls of *T. aphylla* were collected from a mature tree, growing in Mersa-Matrouh, northwest Egypt, in October 1993 and identified by Dr L. Boulos, Prof. of Botany, National Research Centre, Cairo, Egypt.

Isolation and identification. Gall material was extracted with EtOH–H_2O (3:1) The concd extract was applied to a polyamide 6 S CC (Riedel-De Häen AG, Seelze Hanover, Germany) and eluted with H_2O–EtOH mixts of decreasing polarities. The successive eluates were individually dried *in vacuo* and subjected to 2D-PC, whereby 10 different phenolic frs (I–X) were received. Dehydrodigallic acid (**1**, 112 mg), together with dehydrotrigallic acid (**2**, 89 mg) were individually sepd from the 60% aq. EtOH fr. by CC on Sephadex LH-20 using EtOH as solvent, followed by repeated crystallization from acetone–H_2O (4:1) of the individual crude material. Tamarixellagic acid (**3**, 162 mg) was isolated from an acetone extract of the 70% aq. EtOH column fr. also by CC on Sephadex LH-20 and elution with EtOH, followed by EtOH/-$(Me)_2CO$–H_2O (1:1) and then prep. PC using BAW as solvent. Repeated precipitation of the eluted material, thus obtained, from its acetone soln by ether (5 ×) yielded a pure amorphous sample of **3**.

Dehydrodigallic acid (**1**). R_fs and UV spectral data: Table 1. Compound **1** yielded gallic acid on drastic alkaline hydrolysis (2 M aq. NaOH, 100°, 2 hr) and yielded 3,4,5,6,7-pentahydroxyxanthone-1-carboxylic acid (**1a**), mp (uncorr.) 220° on dehydration by conc. H_2SO_4 [5]. ^{1}H NMR of **1**: 7.02 (*d*, J = 2.5 Hz, H-2), 6.5 (*d*, J = 2.5 Hz, H-8$_b$), 6.9 (*s*, H-8). ^{13}C NMR: Table 2. ^{1}H NMR of **1a**: 7.04 (*s*, H-2)*, 7.06 (*s*, H-8)*, *: assignment could be reversed. ^{13}C NMR of **1a**: Table 2.

Dehydrotrigallic acid (**2**). Mp (uncorr.) 265°; R_fs and UV spectral data: Table 1; M_r 506; FAB-MS: neg. ion: 504.8 [M – H]. Compound **2** yielded gallic and dehydrodigallic acids when heated at 100° for 2 hr with 2 M aq. NaOH. Dehydration by conc. H_2SO_4: 37 mg of **2** was heated at 100° for 5 min together with 3 ml conc. H_2SO_4, then left to cool to room temp. The dark reddish soln, thus obtained, was poured gently into 25 ml H_2O. The orange ppt. formed was filtered off, washed thoroughly with H_2O and crystallized (twice) from MeOH to afford 18 mg of the dehydrotrigallic acid xanthone (**2a**). ^{1}H NMR of **2**: 6.42 (*s*, H-14), 6.48 (*d*, J = 2.5 Hz, H-8$_b$), 6.95 (*s*, H-8), 7.02 (*d*, J = 2.5 Hz, H-2); ^{13}C NMR of **2**: Table 2.

Dehydrotrigallic acid xanthone; 3,4,11,12,5,6,7-*heptahydroxybenzpyronoxanthone-1-carboxylic acid* (**2a**). Mp (uncorr.) 245°; R_fs and UV spectral data: Table 1; M_r 470; FAB-MS: neg. ion: 468.8 [M – H]; ^{1}H NMR of **2a**: 6.93 (*s*, H-2)*, 7.0 (*s*, H-8)*, *: assignments may be reversed; ^{13}C NMR of **2a**: Table 2.

2-O-*Galloyl*-3-O-(1-*dehydrodigalloyl*)-4,6-(S)-*hexahydroxybiphenoyl*-$^4C_1$-*glucopyranose* (**3**). R_fs and UV spectral data: Table 1; M_r 954; FAB-MS: neg. ion: 953 [M – H], pos. ion: 955 [M + H]; $[\alpha]_D$ +132° (MeOH; *c* 1.3). Compound **3** yielded glucose, gallic, ellagic and dehydrodigallic acids (co-PC) on complete acid hydrolysis [57 mg of **3** was refluxed with 25 ml, 1.5 M aq. HCl, 100°, 3 hr]. Ellagic acid was filtered off from the cold hydrolysate, and gallic and dehydrodigallic acids were extracted by EtOAc and individually sepd by CC of the EtOAc extract over polyamide, using aq. EtOH (20 and 40%, respectively) as solvent. Gallic acid: R_fs and UV data: identified as given above. Ellagic acid: R_fs and UV spectral data: Table 1; ^{1}H NMR: 7.5 (*s*, H-5, H-5') [4]; ^{13}C NMR: δ 136.4 (C-1$_a$,

C-6$_a$), 140.2 (C-2, C-7), 153.0 (C-3, C-8), 111.4 (C-4, C-9), 107.7 (C-4$_a$, C-9$_a$), 159.2 (C-5, C-10) [13]. Controlled acid hydrolysis of **3** yielded gallic acid (co-PC); 4,6-HHDP-glucose (where HHDP is hexahydroxybiphenoyl) and intermediate **3a** [55 mg of **3** was refluxed together with 25 ml of 0.1 M aq. HCl, 100°, 3 hr]. 4,6-(*S*)-HHDP-glucose: amorphous powder; R_fs and UV spectral data: Table 1; M_r 482; FAB-MS: pos. ion: 483 [M+H]; $[\alpha]_D$ +39.7° (Me_2CO; *c* 0.7). 3-*O*-(1-Dehydrodigalloyl)-(*S*)-4,6-HHDP-(α/β)-$^4C_1$-glucopyranose, (**3a**): R_fs and UV spectral data: Table 1; M_r 802; FAB-MS: neg. ion: 801 [M−H], pos. ion: 803 [M+H]; $[\alpha]_D$ +70° (MeOH; *c* 0.5). Complete acid hydrolysis of **3a** yielded dehydrodigallic acid, ellagic acid and glucose (co-PC). ^{1}H NMR of **3a**: Table 3. ^{1}H NMR of **3**: Table 3. ^{13}C NMR of **3**: glucose moiety: α-anomer: 89.5 (C-1), 72.3 (C-2), 70.4 (C-3), 70.3 (C-4), 69.8 (C-5), 62.5 (C-6); β-anomer: 95.3 (C-1), 72.9 (C-2), 71.5 (C-3), 71.5 (C-4), 65.7 (C-5), 62.5 (C-6); galloyl moiety in both anomers: 118.4 (C-1), 108.1, 108.9 (C-2, C-6), 145.1, 145.2 (C-3, C-5), 138.8, 138.9 (C-4), 165.3, 165.4 (C=O); HHDP moiety in both anomers: 123.7, 124.3 (C-1, C-1′), 105.4, 105.6, 105.7 (C-2, C-2′), 144.1 (C-3, C-3′, C-5, C-5′), 135.3, 135.5 (C-4, C-4′), 115.4, 115.5 (C-6, C-6′), 167.5, 167.6, 167.7 (C=O); dehydrodigalloyl moiety in both anomers: 119.6, 120.0 (C-1), 106.7, 107.7 (C-2), 146.5, 146.6 (C-3), 140.1 (C-4), 147.0, 147.1 (C-4$_a$), 112.6, 112.7 (C-8$_b$), 136.3, 136.6 (C-4$_b$), 142.2, 142.4 (C-5), 142.2 (C-6), 142.4 (C-7), 110.4, 110.6 (C-8), 115.4 (C-8$_b$), 162.3, 162.4 (*t*, *J* = 4.5 Hz in ^{1}H–^{13}C coupled spectrum, esterified C=O) 166.9, 167.0 (C=O of free COOH).

Acknowledgement—The authors are grateful to Dipl. Eng. H. Herzog (Institut für Spektrochemie, Dortmund, Germany) for his constructive co-operation and measurement of NMR spectra.

REFERENCES

1. Ishak, M. S., El-Sisi, H. I., Nawwar, M. A. M. and El-Scherbieny, A. E. (1972) *Planta Med.* **21**, 246.
2. Ishak, M. S., El-Sissi, H. I., El-Scherbieny, A. E. and Nawwar, M. A. M. (1972) *Planta Med.* **21**, 374.
3. Chumbolor, T. K., Bukbulatova, T. N. and Il-Yasova, M. I. (1976) *Khim. Prir. Soedin.* **1**, 131.
4. Nawwar, M. A. M., Buddrus, J. and Bauer, H. (1982) *Phytochemistry* **21**, 1755.
5. Mayer, W. and Fikentscher, R. (1958) *Chem. Ber.* **91**, 1542.
6. Maciel, G. E. and Natter, J. J. (1965) *J. Chem. Phys.* **42**, 2427.
7. Mamblock, H. and Frahm, A. (1980) *Tetrahedron* **36**, 3273.
8. Souleman, A. M. A., Barakat, H. H., El-Mousallamy, A. M. D., Marzouk, M. S. A. and Nawwar, M. A. M. (1991) *Phytochemistry* **30**, 3763.
9. Gupta, R. K., Al-Shafi, S. M., Layden, K. and Haslam, E. (1982) *J. Chem. Soc. Perkin Trans I* 2525.
10. De Bruyn, A., Anteunis, M. and Van Beeumen, J. (1977) *Bull. Soc. Chim. Belg.* **86**, 259.
11. Nawwar, M. A. M., Souleman, A. M., Buddrus, J., Bauer, H. and Linscheid, M. (1984) *Tetrahedron Letters* **25**, 49.
12. Nonaka, G., Sakai, R. and Nishioka, I. (1984) *Phytochemistry* **23**, 1753.
13. Nawwar, M. A. M., Hussein, S. A. M. and Merfort, I. (1993) *Phytochemistry* (submitted).