New Phenolics from *Baccharis* Leaf Exudate

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Several lipophilic phenolics were found in leaf resins of *Baccharis* species, reported earlier to produce terpenoids and flavonoid aglycones. They were identified by NMR and GC/MS studies, respectively. The structure of 2′,4′β-trihydroxy-6′-methoxychalcone from *B. salicifolia* was confirmed by synthesis. Phenylethyl caffeate and benzyl caffeate were found in *B. sarothroides*.

Introduction

A previous study on the leaf resin composition of some *Baccharis* species revealed that the resinous material consists mostly of terpenoids, in which more or less lipophilic flavonoid aglycones are dissolved [1]. In *B. salicifolia,* a major terpenoid was found to be the rare triterpene maniladiol [2]. The major flavonoids were penduletin (6-hydroxykaempferol-3,6,7-trimethyl ether) and pinocembrin, accompanied by five flavones and seven flavonols [1]. One further compound remained unidentified, due to the paucity of material, and was isolated only in a partially purified state (mentioned as compound **6** in [1]. The leaf resin of *B. sarothroides* A. Gray had been found to consist mostly of the trivial triterpene oleanolic acid and the rare diterpene hautriwaic acid and its 2-β-hydroxy derivative [2]. The flavonoid aglycones found in this species were exclusively flavonols, most of them substituted at C-6 and/or C-8, with 5,7,4′-trihydroxy-3,6,8-trimethoxy flavone prevailing [1]. In this material, a blue fluorescent spot (polyamide, UV_{366}) remained unidentified in the earlier study. The two unidentified components from *B. salicifolia* and *B. sarothroides* have now been elucidated by spectroscopic studies. One of these structures was further confirmed by synthesis.

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Materials and Methods

Plant collection data and general procedures for isolation and detection of flavonoids have been reported earlier [1].

Mass spectra were run at 70 eV on a Hewlett-Packard 5985. NMR spectra were recorded on a Bruker WP 200 SY. Derivatized samples were separated and analyzed in a Finnigan 1020 automated GC/MS (incorporating a Data General Nova 3 computer) [3].

Synthesis of **I**. Phloracetophenone-4-O-neohesperidoside (**II**) (1 g, 0.002 mol) was dissolved in dry DMF (10 ml), anhydrous K_2CO_3 (550 mg, 0.004 mol) and JMe (710 mg, 0.005 mol) were added and the mixture was stirred at 60–70 °C for 6 h. After usual work-up the crude product was found, by ^{1}H NMR, to be a mixture of **III**, dimethylated **II**, and **II** in a relative ratio of approximately 3:1.5:1. This mixture was dissolved in 10% HCl (75 ml) and kept at 80–90 °C for 1 h. After cooling, a white powder was collected and purified by CC in order to eliminate undesired hydrolysis products. We thus obtained 2,4-dihydroxy-6-methoxy-acetophenone **IV** (105 mg), m.p. 195–197 °C (EtOAc–hexane). MS (DIP) *m/z* (rel. int.): 182 (M^+, 37), 167 (M^+-CH_3, 100), 152 ($M^+-2\times CH_3$, 13), 124 (9), 69 (31). ^{1}H NMR

($CDCl_3$) ppm: 5.96 (1H, d; J = 2 Hz), 5.93 (1H, d; J = 2 Hz), 3.87 (3H, s; OCH_3), 2.60 (3H, s; $COCH_3$).

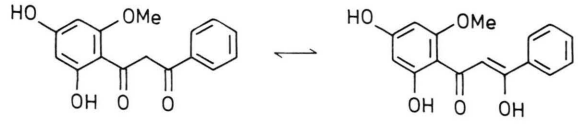
To a solution of **IV** (100 mg) in 1.5 M NaOH (2.5 ml) benzoyl chloride (210 mg) was added and the mixture was stirred at room temperature for 2 h. After cooling, it was neutralized with 1 N HCl, extracted with ether and the crude product was purified by CC. 2,4-Dibenzoyloxy-6-methoxy-acetophenone **V** was obtained as a colourless oil (40 mg). MS m/z (rel. int.): 390 (M^+, 28), 375 (M^+-CH_3, 4), 359 (M^+-OCH_3, 2), 105 (Ph–C=O, 100), 77 (Ph, 53). ^1H NMR ($CDCl_3$) ppm: 8.20 (2H, dd; J = 8.5 and 1.5), 8.13 (2H, dd; J = 8.5 and 1.5), 7.58 (6H, m), 6.87 (1H, d; J = 2 Hz), 6.81 (1H, d; J = 2 Hz), 3.91 (3H, s; OCH_3), 2.54 (3H, s; $COCH_3$).

V (40 mg) was dissolved in dry DMSO (1 ml) and powdered NaOH (320 mg) added. The mixture was stirred at room temperature for 5 min, then poured onto ice (25 g), extracted with EtOAc, washed, dried and purified by CC to yield 20 mg of a light-yellow product, m.p. 167–169 °C, which is identical with natural **I** in every respect.

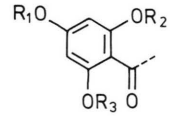
GC/MS data indicated two phenolic compounds to be present in approximately equal quantities in the *B. sarothroides* fraction exhibiting the blue fluorescent spot on TLC. Comparison of their retention times and mass spectra with those of authentic reference compounds enabled their identification as benzyl *trans*-caffeate and phenylethyl *trans*-caffeate (as the respective bis-TMS derivatives, M^+ m/z 414 and 428).

Results

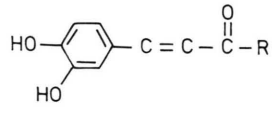
The unknown constituent **I** from *B. salicifolia* was isolated in pure state after repeated preparative TLC on silica (toluene/methylethylketone 9:1). It formed light-yellow needles, m.p. 168–170 °C. UV λ_{max}^{MeOH} (235), (245), 282, 368 nm; + $AlCl_3$ (235), 310, 393 nm. For MS and NMR data see Fig. 1 and Table I, respectively. From its UV spectrum and fragmentation pattern in the MS it was concluded that **I** is a polyhydroxydibenzoylmethane. The base peak at m/z 105 indicates that one benzene ring is unsubstituted, while the other bears two hydroxyls and 1 methoxy group. The latter is placed at C-6′ on the basis of two doublets (J = 2 Hz) for H-3′ and H-5′, demonstrating asymmetric substitution of ring A. The product is thus identified as 1-(2,4-dihydroxy-6-methoxyphenyl)-3-phenyl-1,3-propanedione (2′,4′,β-trihydroxy-6′-methoxy chalcone). The ^1H NMR spectrum in acetone-d_6 revealed that in solution there exists an equilibrium of keto (**Ia**) and enol (**Ib**) tautomeric forms in a relative ratio of 7:3.



Ia Ib



	R_1	R_2	R_3
II	Neohesperidose	H	H
III	Neohesperidose	Me	H
IV	H	Me	H
V	-C-Ph (=O)	Me	-C-Ph (=O)

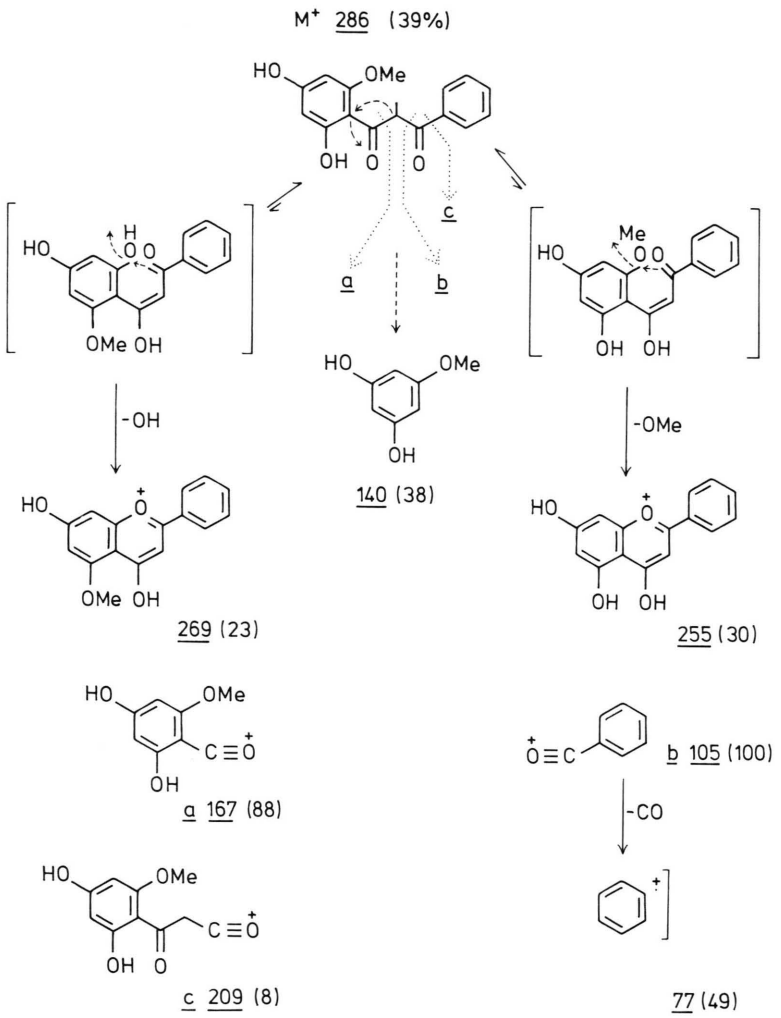


VI R=-O-CH_2-Ph
VII R=-O-CH_2-CH_2-Ph

The assignment of this structure was confirmed by synthesis in a five step reaction sequence with 7% overall yield. Phloracetophenone-4-O-neohesperidoside, which is easily obtained from naringin by a well-known method [4], was selectively methylated; then the sugar moiety was eliminated by acidic hydrolysis and the aglycone dibenzoylated. Mild alkaline treat-

Table I. ^1H NMR data of **Ia** and **Ib** (200 MHz, acetone-d_6, TMS as internal standard).

	Diketonic **Ia**	Keto-enolic **Ib**
3′-H	5.93, d J = 2	5.97, d J = 2
5′-H	6.00, d J = 2	6.10, d J = 2
2″,6″-H	8.03, dd J = 8.5; 1.5	7.95, dd J = 8.5; 1.5
3″,4″,5″-H	7.55, m	7.58, m
2-H	4.60, s	7.46, s
2′-OH	13.72, s	15.53, s
4′-OH	9.50, bs	9.50, bs
β-OH	–	13.12, s
OMe	4.00, s	4.00, s



ment of the reaction product allowed Baker-Venkataraman rearrangement and cleavage of the second benzoyl moiety without further cyclization. The final product is identical with natural **I** in every respect.

From some fractions of *B. salicifolia,* a colourless product crystallized, which was not detected in our usual TLC systems, *i.e.* it is not visible in UV_{366} and UV_{254} before and after spraying with Naturstoff-reagenz A, nor does it react with $SbCl_3$ and $MnCl_2$ spray reagent. In the MS it exhibits M^+ at *m/z* 386 ($C_{23}H_{46}O_4$). The ^1H NMR spectrum is very clear, thus showing that the product is pure. Interpretation of the NMR signals allows its identification as a monoglyceride, namely 1-mono-eicosanyl-glycerol (glycerol ester of arachidic acid).

The product causing the blue fluorescent spot on TLC of *B. sarothroides* leaf exudate could not be isolated, due to the minute amount present; it was obtained only in a mixed fraction containing some flavonoids. In this case, structure elucidation was possible by GC/MS analysis and comparison of data with those obtained during recent research on phenolic acid esters from poplar bud exudates and from propolis or bee-glue (*cf.* [5]). It thus became evident that the fluorescent spot is caused by benzyl caffeate and phenylethyl caffeate.

It should be mentioned in this context that the rare 5,7,4′-trihydroxy-3,6,8-trimethoxyflavonol, reported earlier from *B. sarothroides* [1], has now been confirmed by direct comparison with an authentic marker, isolated from *Gutierrezia microcephala* [6].

Discussion

The β-hydroxychalcone or dibenzoylmethane **I** found in *Baccharis salicifolia* certainly is the most interesting of the phenolics now identified. To our knowledge it is a novel natural product. Further representatives of this unusual group of flavonoids have been reported previously from *Milletia ovatifolia* [7], *Glycyrrhiza echinata* [8], *Lonchocarpus costaricensis* [9], *Polygonum nepalense* [10], *Primula pulverulenta* [11], and *Tephrosia procumbens* [12]. A product that is closely related structurally to the β-hydroxychalcone **I** from *B. salicifolia,* has been found earlier in *B. bigelovii,* namely 2β,5,7-trihydroxyflavanone [13], which can be considered the cyclic form of 2′,4′,6′-trihydroxy-dibenzoylmethane. The 7-glucoside of this unusual flavanone had been reported from *Malus* leaves [14], and the 7-methyl ether was found in *Populus* bud exudates [15].

The two blue fluorescent products from *B. sarothroides,* phenylethyl caffeate and benzyl caffeate, are known as characteristic constituents of the bud exudate of poplar species [5] and they are hence encountered in propolis, too [16].

In a previous report and review on flavonoid aglycones from *Baccharis* [1] we emphasized that reinvestigations of the species studied earlier by various authors would probably yield numerous additional flavonoids if these investigations were done with leaf exudates. The present results prompt us to stress that such reinvestigations would certainly be well worthwhile as they would reveal new products and rare structures.

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