A New Lignan from Brazilian Propolis

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The known flavonoid kaempferid **4** and a new benzofurane lignan **1** were isolated from Brazilian propolis. The new lignan is a suitable taxonomic marker and gives new possibilities for identification of propolis plant sources in the tropical regions.

Introduction

Propolis (bee glue) is a resinous material collected by honey-bees from different plant exudates and used as a general purpose sealer in the construction of the hive. It is known to possess a broad spectrum of biological activities: antibacterial, antiviral, fungicidal, anti-inflammatory, antitumor, immunomodulatory, local anaesthetic etc. (Marcucci, 1995). The bud exudates of different poplar species are the main sources of bee glue in the temperate zone incl. Europe, Asia and North America (Greenaway *et al.*, 1987, Bankova *et al.*, 1992, Wollenweber *et al.*, 1987). For this reason the chemical composition of propolis samples originating from these regions is characterized by the same main components: flavonoids and esters of substituted cinnamic acids (Marcucci, 1995). The origin and chemistry of tropical propolis is still an open question, and only few communications have been published on the chemical composition and possible plant sources of bee glue from Brazil and Venezuela (Aga *et al.*, 1993, Tomas-Barberan *et al.*, 1993, Bankova *et al.*, 1995). In this work, we report the isolation of a new lignan from Brazilian propolis. Till now, different phenolic compounds have been found in propolis but this is the first isolation of a lignan from it.

Experimental

Propolis was collected in Brazil, Parana State, near Prudentopolis.

Extraction of propolis

Propolis (20 g) was cut into small pieces and extracted with boiling MeOH (200 ml) for 2 h. The extract was filtered hot, diluted with water (150 ml) and extracted successively with hexane (3 x 350 ml) and Et_2O (350 ml). The ether extract was evaporated *in vacuo* to dryness (7.1 g).

Isolation of individual compounds

The ether extract was subjected to column chromatography on silica gel and eluted with hexane-acetone gradient to produce several fractions. After repeated column chromatography on silica gel with hexane-acetone, chloroform-ethyl acetate and methylene chloride-methanol, **1** and **4** were isolated.

2,3-dihydro-2-(4-hydroxy-3-methoxyphenyl)-5-(3-acetyloxy-(*E*)-propenyl)-3-acetyloxymethyl-benzofurane **1**, 16 mg. $[\alpha]_D^{20}$ +0.0 (*c*=0.31, $CHCl_3$); UV (MeOH) λ_{max} : 217, 286. ^{1}H-NMR ($CDCl_3$): δ 2.02 (3H, s, OAc), 2.10 (3H, s, OAc), 3.83 (1H, m, H-3), 3.86 (3H, s, 3'-OMe), 3.90 (3H, s, 3-OMe), 4.33 (2H, ddd, *J*=5.5, 7.7, 11.1 Hz, CH_2-11), 4.71 (2H, dd *J*=6.6, 1.1 Hz, CH_2-11), 5.48 (1H, d *J*=7.4 Hz, H-2), 6.15 (1H, dt *J*=15.8, 6.6, H-9), 6.65 (1H, d *J*=15.8 Hz, H-8), 6.80–6.92 (5H, m, aromatics). ^{13}C-NMR ($CDCl_3$): See Table I. MS (EI 70 eV) *m/z:* 442 (M^+, 2%); 368 $[(M\text{-}AcO\text{-}CH_3)^+$, 3%]; 322 $[(M\text{-}2AcOH)^+$, 5%]; 123 (10%), 43 (100%).

3,5,7-Trihydroxy-4'-methoxyflavone (**4**) (kaempferid), 21 mg, yellow crystals. UV in MeOH and with shift reagents identical with the data for kaempferid in Mabry *et al.* (1970); ^{13}C-NMR identical with the spectrum of kaempferid in

Markham *et al.* (1982), mass and ^{1}H-NMR spectra identical with the data in Popravko *et al.* (1969).

Results and Discussion

The ether fraction from the methanolic extract of the investigated propolis sample was subjected to repeated column chromatography on silica gel and afforded 10,11-di-O-acetyl dehydrodiconiferyl alcohol (**1**). This substance has a molecular ion peak at *m/z* 442. The ^{1}H-NMR spectrum showed five aromatic protons; two *trans*-olefinic protons which appeared as an AB part of an ABX_2 system; a 2,3-disubstituted 2,3-dihydrobenzofurane ring; one OMe group and two OAc groups. The ^{13}C-NMR spectrum showed, apart from the resonances of the acetyl and methoxyl groups, signals corresponding to 18 carbon atoms, consistent with a lignan structure. The analysis of the ^{1}H-NMR spectrum, ^{1}H-^{1}H correlation spectroscopy (^{1}H-^{1}H COSY), ^{13}C-NMR and DEPT spectral data enabled us to construct the complete structure of **1**. The assignment of the signals in the ^{13}C-NMR spectrum based on the literature precedents (Luedermann and Nimtz, 1974; Yoshikava *et al.*,1995) are given in Table I. For ^{1}H-NMR data see Experimental. The shift of the protons at C-10 and C-11 in **1**, compared to the corresponding signals in similar lignans with free OH groups at the same positions (e.g. **2**, Luedermann and Nimtz, 1974), indicated that these hydroxyl groups were acetylated. Consequently, compound **1** is 10,11-di-O-acetyl dehydrodiconiferyl alcohol. The relative stereochemistry of the dihydrofurane ring was elucidated by NOE experiments, as it was done for similar compounds (Yoshikava *et al.*,1995). The strong NOE-s between H-3 and H-2'/6', and the very weak NOE between H-2 and H-3 indicated *trans* 2/3 configuration, as in the most known dihydrobenzofurane lignans.

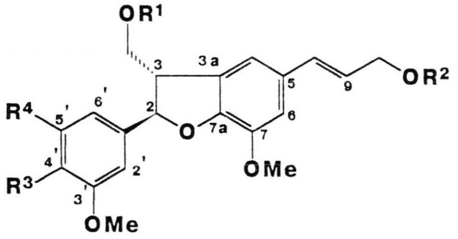
Table I. ^{13}C-NMR data of 10,11-diacetyl dehydrodiconiferyl alcohol (**1**), dehydrodiconiferyl alcohol (**2***) and 2,3-dihydro-3-acetyloxymethyl-5(3-glucosyloxypropenyl)-7-methoxy-2-(3',4',5'-trimethoxyphenyl)benzofurane (**3****).

C-atom	1	2*	3**
2	88.8	88.3	89.5
3	50.3	54.7	51.7
3a	127.7	132.0	129.0
4	115.3	116.2	116.3
5	130.5	130.4	132.6
6	110.6	111.8	112.3
7	144.4	145.1	145.6
7a	146.7	148.9	149.2
8	134.4	130.9	133.8
9	121.1	128.0	124.7
10	65.3	63.3	70.7
11	65.2	64.6	66.6
Ac	170.8***	-	172.5
	20.8***	-	20.8
OMe	56.0	56.4	56.7
1'	132.2	134.3	138.2
2'	108.6	110.7	104.2
3'	148.3	148.6	154.6
4'	145.9	147.3	138.8
5'	114.3	115.8	154.6
6'	119.5	119.5	104.2
OMe	56.0	56.4	56.7
	-	-	56.7
	-	-	61.1

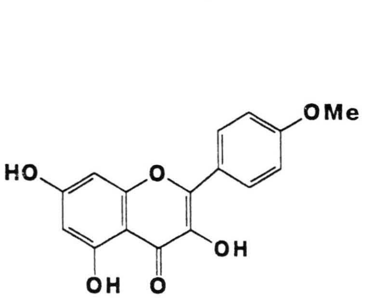
* Data from Luedermann and Nimtz (1974) in Me_2CO-d_6-D_2O (9:1).

** Data from Yoshikava *et al.*,(1995) in CD_3OD.

*** Identical for both acetyl groups.



1. $R^1 = R^2 = Ac$, $R^3 = OH$, $R^4 = H$
2. $R^1 = R^2 = R^3 = OH$, $R^4 = H$
3. $R^1 = Ac$, $R^2 = Glc$, $R^3 = R^4 = OMe$



4

Fig. 1.

The lignan **1** has not been reported before in nature, although the desacetyl compound, dehydrodiconiferyl alcohol **2**, was known long ago (first isolated from *Silybum marianum* by Weinges *et al.* (1970)). Salama *et al.* (1981), isolated a 4-β-D-glucoside of **2** but the configuration of the dihydrofurane ring in this lignan was reported to be 2/3 *cis* without any discussion.

Along with **1** we isolated from the sample investigated a flavonoid which was identified as kaempferid (4'-O-methyl-kaempferol) **4** by comparing its MS, UV, ^{1}H-NMR and ^{13}C-NMR spectra with literature data (Mabry *et al.*, 1970, Markham *et al.*, 1982; Popravko *et al.*, 1969). This flavonoid was found earlier in propolis from the European part of Russia and was shown to originate from the bud exudate of birch (*Betula* spp.) (Popravko, 1976).

The origin of the phenolic compounds **1** and **4** in Brazilian propolis is unclear. The identification of the new natural compound **1** in this sample provides a suitable taxonomic marker because the isolation of **1** from some plant exudate will be an indication that this exudate is one of the sources of Brazilian propolis.

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