Pergamon

0031-9422(94)00718-7

Phytochemistry, Vol. 38, No. 3, pp. 751–753, 1995

0031–9422/95 \$9.50 + 0.00

CINNABARONE, A BIFLAVONOID FROM DRAGON'S BLOOD OF *DRACAENA CINNABARI*

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(*Received* 5 *July* 1994)

Key Word Index—*Dracaena cinnabari*; Agavaceae; resin; biflavonoid; cinnabarone.

Abstract—A new biflavonoid, cinnabarone, was isolated from dragon's blood of *Dracaena cinnabari*. Its structure was established, mainly by NMR spectroscopy, as a dihydrochalcone linked by a carbon–carbon bond to a deoxotetrahydrochalcone.

INTRODUCTION

In continuation of our work [1] on the constituents of dragon's blood from *Dracaena cinnabari* Balf. fil., we isolated a biflavonoid of a novel structure type, for which the name cinnabarone is proposed. Its structure was determined unambiguously as **1** mainly on the basis of NMR spectroscopy as outlined below.

RESULTS AND DISCUSSION

The elemental composition of **1** was shown by high resolution MS to be $C_{32}H_{32}O_7$. The base peak at *m/z* 377 was formed by cleavage of the CH-C(α') bond (benzyl cleavage). Further diagnostic fragments were detected at *m/z* 272 (**a**), 256 (**b**) [splitting into the two monomers], *m/z* 137 [cleavage of the C(α')-C(β') bond, benzyl cleavage] and *m/z* 121 [cleavage of the carbonyl-C(α) bond, ketone α-cleavage].

1D and 2D NMR methods, including inverse technique, were used to determine the structures of **1** and **2**, and to provide the assignments. The methods applied were ^{13}C attached proton test; 2D ^1H–^1H COSY-90; 2D ^1H–^1H delayed COSY-45 [2, 3]; 2D ROESY [4]; proton detected heteronuclear chemical shift correlation via $^1J(^{13}C–^1H)$ [HMQC] [5] and heteronuclear multiple bond connectivity (HMBC) [6] experiments. The signals of the two compounds showed proton and carbon shifts in a very narrow spectral region (Tables 1 and 2). Nevertheless, the proton–proton coupling networks and the proton–carbon assignments via $^1J(^{13}C–^1H)$ could be analysed by ^1H–^1H COSY-90 and HMQC technique. Only two singlets were present.

The major problem in the assignments of **1** and **2** was to detect the connectivity of the aromatic substituents with the aliphatic side-chains. This was possible by 2D ^1H–^1H delayed COSY-45 technique which allowed the determination of ^1H–^1H-coupling patterns via $^4J(^1H–^1H)$ and $^5J(^1H–^1H)$. Long-range couplings were found for **1** between the protons CH (δ = 4.15 ppm) and H-6 (δ = 6.89 ppm), H-2‴, H-6‴ (δ = 7.02 ppm), between H-β (δ = 2.87 ppm) and H-6 (δ = 6.89 ppm), between H-β' (δ = 2.38 ppm) and H-6″ (δ = 6.79 ppm), between H-3 (δ = 6.36 ppm) and H-6 (δ = 6.89 ppm) [*para*-position], as well as between H-3″ (δ = 6.35 ppm) and H-5″ (δ = 6.26 ppm) [*meta*-position].

The connectivities of the four aromatic rings were unambiguously determined by analysing the NOEs of **1** using ROESY experiments. Besides the expected NOEs between CH/H-2‴, H-6‴; H-β/H-6 and H-β'/H-6″, strong NOEs were found between the methoxy groups and CH, H-3, H-3″ and H-5″, as well as between H-α/H-2′ and H-6/H-2‴, H-6‴. These data proved the connectivity of the aromatic rings, as well as the 4- and 4″-position of methoxy. We used proton-detected multiple bond ^1H–^{13}C correlation (HMBC) of **1** to obtain an assignment of all quaternary carbons and to prove the connectivity by an independent way. We found all expected correlations via $^2J(^{13}C–^1H)$ and $^3J(^{13}C–^1H)$ according to structure **1**. The connectivity of the carbonyl group was only determined by the found correlations of C=O carbon with the protons H-α, H-β, H-2′ and H-6′. The ^1H NMR spectrum of the acetate **2** proved the existence of four acetyl groups (corresponding to four hydroxyl groups in **1**). From these data structure **1** is established for this new biflavonoid which is composed of a dihydrochalcone and a deoxotetrahydrochalcone moiety connected by a C–C bond.

EXPERIMENTAL

NMR experiments were carried out on a Varian UNITY 500 spectrometer operating at 499.85 (^1H) and 125.7 MHz (^{13}C). CD_3OD (**1**) and $CDCl_3$ solns (**2**) of

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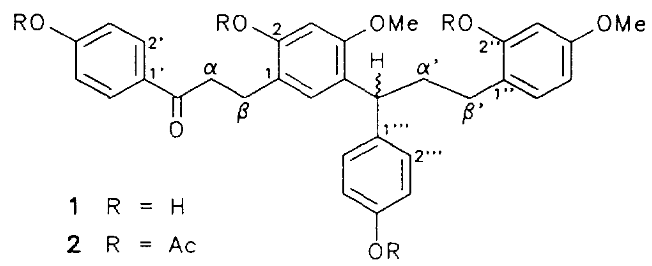

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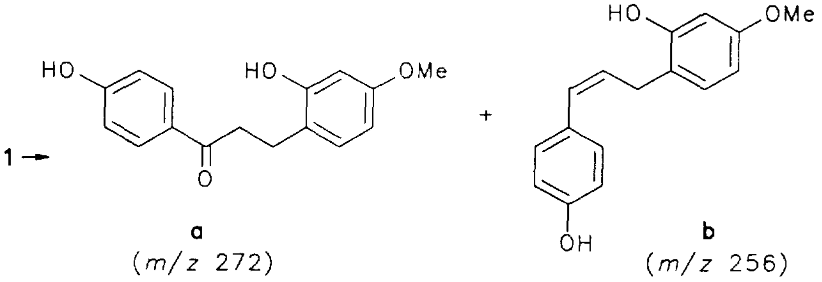
2 R = Ac



a (m/z 272)

b (m/z 256)

Table 1. ^{1}H NMR chemical shifts (ppm) and ^{1}H–^{1}H-coupling constants (Hz) of **1** and **2**

H	**1** (CD_3OD)	**2** ($CDCl_3$)
α	3.08 (2H, *t*, 7.1 Hz)	3.18 (2H, *t*, 7.0 Hz)
β	2.87 (2H, *t*, 7.1 Hz)	2.97 (2H, *t*, 7.0 Hz)
α′	2.03 (2H, *m*)	2.09 (2H, *m*)
β′	2.38 (2H, *m*)	2.51 (2H, *t*, 6.8 Hz)
CH	4.15 (1H, *t*, 7.9 Hz)	3.96 (1H, *t*, 7.6 Hz)
3	6.36 (1H, *s*)	6.54 (1H, *s*)
6	6.89 (1H, *s*)	7.11 (1H, *s*)
2′, 6′	7.81 (2H, *d*, 6.8 Hz)	7.97 (2H, *d*, 7.0 Hz)
3′, 5′	6.76 (2H, *d*, 6.8 Hz)	7.17 (2H, *d*, 7.0 Hz)
3″	6.35 (1H, *d*, 2.4 Hz)	6.50 (1H, *d*, 2.5 Hz)
5″	6.26 (1H, *dd*, 7.9; 2.4 Hz)	6.57 (1H, *dd*, 7.8; 2.5 Hz)
6″	6.79 (1H, *d*, 7.9 Hz)	6.99 (1H, *d*, 7.8 Hz)
2‴, 6‴	7.02 (2H, *d*, 7.0 Hz)	6.97 (2H, *d*, 7.2 Hz)
3‴, 5‴	6.64 (2H, *d*, 7.0 Hz)	7.15 (2H, *d*, 7.2 Hz)
4-OMe	3.74[a] (3H, *s*)	3.73[a] (3H, *s*)
4″-OMe	3.75[a] (3H, *s*)	3.75[a] (3H, *s*)
2-Ac	—	2.15[b] (3H, *s*)
4′-Ac	—	2.25[b] (3H, *s*)
2″-Ac	—	2.27[b] (3H, *s*)
4‴-Ac	—	2.30[b] (3H, *s*)

[a, b] May be reversed.

20 mg of **1** and **2** in 0.5 ml of solvent were used. Chemical shifts were referenced to int. TMS ($\delta = 0$ ppm), CD_3OD ($\delta = 49.0$ ppm) and $CDCl_3$ ($\delta = 77.0$ ppm) for ^{1}H and ^{13}C NMR, respectively. The delay τ_1 in HMQC and HMBC was adjusted to ^1J (^{13}C–^{1}H) = 150 Hz. The delay τ_2 in HMBC was set to 70 msec according to long-range coupling *ca* 7 Hz and to 140 msec according to long-range coupling *ca* 3 Hz.

Plant material. Dragon's blood from *Dracaena cinnabari* was collected in Socotra Island of Yemen in summer 1992. A voucher specimen of resin is deposited at the Institute of Plant Biochemistry, Halle.

Table 2. ^{13}C chemical shifts (ppm) of **1** in CD_3OD

C	**1**	C	**1**
α	40.0	1″	124.8
β	27.3	2″	159.6[b]
α′	37.2	3″	99.8
β′	29.5	4″	157.3[b]
CH	43.1	5″	107.5
1	123.2	6″	131.1
2	155.9[a]	1‴	130.1
3	99.7	2‴, 6‴	130.0
4	157.5[b]	3‴, 5‴	115.7
5	120.8	4‴	155.1[a]
6	130.1	C=O	202.1
1′	138.3	4-OMe	55.6[c]
2′, 6′	132.0	4″-OMe	55.7[c]
3′, 5′	116.2		
4′	163.6		

[a–c] May be reversed.

Cinnabarone (**1**). The powdered resin was successively extracted with *n*-hexane, $CHCl_3$ and MeOH. Evapn of MeOH *in vacuo* gave a residue, which was chromatographed on silica gel (Merck 60, 0.063–0.2 mm). Elution with $CHCl_3$–MeOH (95:5) gave **1**, which was rechromatographed on silica gel with toluene–EtOAc–HOAc (70:30:1) and obtained as a powder (yield 0.66%). $[\alpha]_D^{22}$ $\pm 0.0°$ (MeOH; *c* 0.40), R_f 0.26 [silica gel 60 F_{254}; toluene–EtOAc–HOAc (60:30:2)]. UV λ_{max}^{EtOH} nm (log ε): 207 (4.48), 221sh (4.25), 280 (4.05). IR ν_{max}^{KBr} cm^{-1}: 3350 (OH), 1650 ($>C=O$), 1593, 1500. EIMS (70 eV) m/z (rel. int.): 528.2169 $[M]^+$ ($C_{32}H_{32}O_7$ calcd 528.2148) (25), 377.1380 ($C_{23}H_{21}O_5$, calcd 377.1389) (100), 272.1034 $[\mathbf{a}]^+$ ($C_{16}H_{16}O_4$, calcd 272.1049) (7), 256.1108 $[\mathbf{b}]^+$ ($C_{16}H_{16}O_3$, calcd 256.1099) (27), 137.0617 ($C_8H_9O_2$, calcd 137.0602) (64), 121.0320 ($C_7H_5O_2$, calcd 121.0290) (64).

Tetraacetate (**2**). EIMS (70 eV) m/z (rel. int.): 696 $[M]^+$ (23), 654 $[M - CH_2CO]^+$ (62), 612 $[M - 2\ CH_2CO]^+$

(6), 462 (30), 461 (100), 419 (50), 377 (5), 340 (14), 298 (23), 256 (9), 241 (14), 151 (11), 137 (17), 121 (14), 107 (6).

Acknowledgements—We are grateful to Dr J. Schmidt for MS measurements and to the Bundesministerium für Forschung und Technologie, Bonn, for financial support.

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