RUMBRIN, A NEW CYTOPROTECTIVE SUBSTANCE PRODUCED BY *Auxarthron umbrinum*

II. PHYSICO-CHEMICAL PROPERTIES AND STRUCTURE DETERMINATION

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The structure of rumbrin (Fig. 1), a new cytoprotective substance, was elucidated by NMR spectral analysis. Rumbrin was found to possess a novel skeleton containing α-pyrone, tetraene and pyrrole moieties.

In the preceding paper[1], we described the fermentation, isolation and biological activities of rumbrin, which is produced by *Auxarthron umbrinum*. This paper describes the physico-chemical properties and structural studies on rumbrin.

Rumbrin (**1**) is a red crystalline solid with the properties listed in Table 1. The molecular formula of **1** was determined to be $C_{20}H_{20}NO_3Cl$ by HRFAB-MS ($(M+H)^+$ *m/z* cacld: 358.1210, found: 358.1206).

Fig. 1. The total structure of rumbrin.

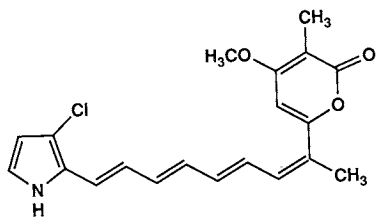


Table 1. Physico-chemical properties of rumbrin.

Appearance	Red needles
MP (dec.)	170~171°C
Molecular formula	$C_{20}H_{20}NO_3Cl$
HRFAB-MS Calcd:	358.1210
Found:	358.1206 $(M+H)^+$
UV λ_{max} nm (ε) (in MeOH)	269 (12,750), 341 (17,350), 442 (33,800)
IR *v* (KBr) cm^{-1}	3420, 2920, 1660, 1620, 1525, 1240
Rf value[a]	0.46
Soluble	MeOH, DMSO, $CHCl_3$, EtOAc
Insoluble	H_2O, hexane

[a] Solvent system: $CHCl_3$ - MeOH (50 : 1) Kieselgel 60 F_{254}.

Fig. 2. ^1H NMR spectrum of rumbrin in DMSO-d_6 (500 MHz).

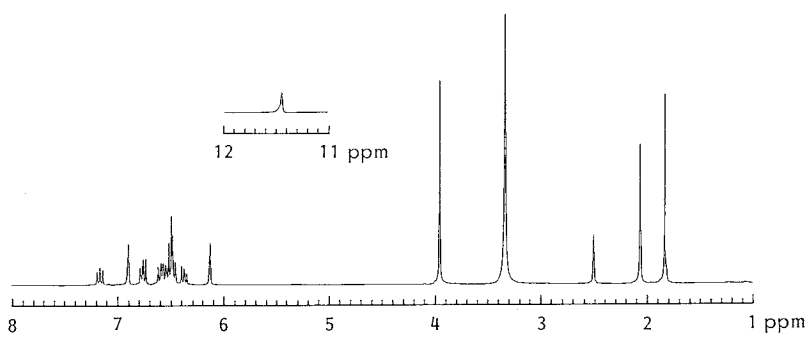
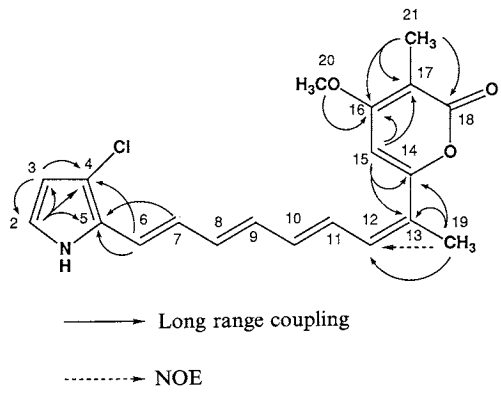


Table 2. The 500 MHz ^{1}H NMR and 125 MHz ^{13}C NMR spectral data for rumbrin[a].

Position	δ_H	δ_C	Position	δ_H	δ_C
1-NH	11.44 (dd 2.3, 2.6)[b]		12	6.47 (d 12.5)	134.7 (d)
2	6.90 (dd 2.6, 3.0)	120.2 (d)	13		124.8 (s)
3	6.13 (dd 2.3, 3.0)	109.0 (d)	14		159.1 (s)
4		111.5 (s)	15	6.50 (s)	96.2 (d)
5		125.9 (s)	16		165.7 (s)
6	6.49 (d 15.0)	120.2 (d)	17		100.3 (s)
7	6.75 (dd 11.0, 15.0)	124.8 (d)	18		163.4 (s)
8	6.59 (dd 11.0, 14.5)	135.9 (d)	19	2.05 (s)	21.1 (q)
9	6.37 (dd 11.3, 14.5)	131.3 (d)	20	3.95 (s)	56.6 (q)
10	6.54 (dd 11.3, 14.0)	137.9 (d)	21	1.92 (s)	8.5 (q)
11	7.16 (dd 12.5, 14.0)	128.9 (d)			

[a] Taken in DMSO-d_6.
[b] Coupling constants in J=Hz.

Fig. 3. ^{1}H-^{13}C long range couplings and NOE.



The ^{1}H NMR spectrum of **1** (Fig. 2) showed 14 signals, which were attributed to two singlet CH_3 (δ_H 1.92 and 2.05), one OCH_3 (δ_H 3.95), one imine (δ_H 11.44) and 10 olefinic methine protons.

The ^{13}C NMR spectrum of **1** showed signals for 20 carbons. The distortionless enhancement by polarization transfer (DEPT) experiment assigned them to 3 methyl, 10 sp^2 methine, and 7 quaternary carbons including one ester carbonyl carbon (C-18) and 2 oxygenated sp^2 carbons (C-14 and C-16).

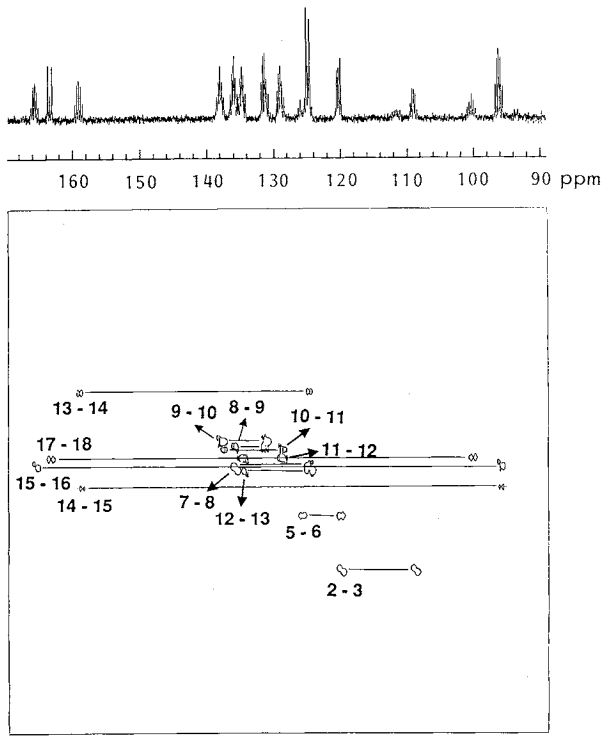
The ^{1}H-^{1}H COSY spectrum established a tetraene structure composed of C-6~C-12 with *E* geometrical configurations for C-6, 8 and 10 apparent from the coupling constants ($J_{6,7}$=15.0 Hz, $J_{8,9}$=14.5 Hz and $J_{10,11}$=14.0 Hz).

The HMBC experiment on **1**[2] showed long range couplings of 19-CH_3 to C-13 (δ_C 124.8) and C-14 (δ_C 159.1), 21-CH_3 to C-16 (δ_C 165.7), C-17 (δ_C 100.3) and C-18 (δ_C 163.4), 20-OCH_3 to C-16 and 15-H to C-13, C-14, C-16 and C-17. These correlations established the connectivities of C-13~C-18. Taking into consideration the number of oxygen atoms contained in **1** and the chemical shifts of C-14 and C-18, one oxygen atom must be inserted between C-14 and C-18. Thus, the existence of an α-pyrone unit in **1** was confirmed as shown in Fig. 3.

The HMBC experiment also showed long range couplings of 19-CH_3 to C-12 (δ_C 134.7), C-13 (δ_C 124.8) and C-14. Thus, the tetraene and the α-pyrone units are linked through C-13 (Fig. 3). The diagonostic ^{13}C chemical shift for C-19 (δ_C 21.1)[3] and NOE between 12-H and 19-CH_3 defined the configuration of the C-12~C-13 double bond as *Z*.

In the ^{1}H-^{1}H COSY spectrum, cross peaks were observed among the two methine protons (2-H (δ_H 6.90, J=2.6, 3.0 Hz) and 3-H (δ_H 6.13, J=2.3, 3.0 Hz)) and an imine proton (δ_H 11.44, J=2.3, 2.6 Hz). In addition, long range couplings were observed from 3-H to C-2 (δ_C 120.2) and C-4 (δ_C 111.5), 2-H to C-3 (δ_C 109.0), C-4 and C-5 (δ_C 125.9), 6-H to C-4 and C-5, and 7-H to C-5 in the HMBC experiment.

Fig. 4. 2D INADEQUATE spectrum of [1,2-$^{13}C_2$]acetate labeled rumbrin.



These couplings indicated the presence of a 2,3-disubstituted pyrrole ring consisting of C-2 ~ C-5, and the linkage to the tetraene unit at C-5. Therefore, attachment of the chlorine atom to the quaternary carbon C-4 was assigned (Fig. 3). Based on all these findings, the total structure of **1** was established to be (1*Z*,3*E*,5*E*,7*E*)-6-(8-(3-chloro-1*H*-pyrrol-2-yl)-1-methyl-1,3,5,7-octatetraenyl)-4-methoxy-3-methyl-2*H*-pyran-2-one (Fig. 1).

In the ^{1}H-^{13}C COSY spectrum, the olefinic carbon signals could not be unambiguously assigned because of the overlapping of their proton signals. Therefore, we assigned these carbons and confirmed the structure of **1** using $^{1}J_{C-C}$ information. The biosynthetic origin of the polyene and α-pyrone units was expected to be mainly acetate[4]. Thus, an incorporation experiment with [1,2-$^{13}C_2$]acetate was carried out with a culture of *A. umbrinum* n13. By adding 1 g of sodium [1,2-$^{13}C_2$]acetate 48 hours after the beginning of the 1 liter culture, 4 mg of labeled **1** was obtained. A 2D INADEQUATE experiment using this sample confirmed the total structure of **1** and the assignments of all sp^2 carbons (Fig. 4).

Experimental

General

Mass spectra were measured on a VG Analytical ZAB-HF in the FAB mode using a *meta*-nitrobenzylalcohol matrix. UV spectra were recorded using a Hitachi U-3200 spectrophotometer. NMR spectra were obtained on a JEOL JNM-GX500 spectrometer with ^{1}H NMR at 500 MHz and ^{13}C NMR at 125 MHz. Chemical shifts are given in ppm using TMS as internal standard.

Labeled Compound

Sodium [1,2-$^{13}C_2$]acetate was purchased from MSD Isotopes.

Incorporation Studies

The incorporation studies were conducted as follows: The strain n13 was inoculated into 100 ml of a seed medium consisting of glucose 2.0%, peptone 0.5%, KH_2PO_4 0.5%, $MgSO_4 \cdot 7H_2O$ 0.05%, $FeSO_4 \cdot 7H_2O$ 0.05% and agar 0.1% in a 500-ml Erlenmeyer flask (pH 6.0), and cultured at 25°C for 5 days on a rotary shaker (180 rpm). Three ml of this seed culture was inoculated into 100 ml of the production medium having the same composition as the seed medium in 500-ml Erlenmeyer flasks. After incubation at 25°C for 2 days on the rotary shaker, 100 mg of sodium [1,2-$^{13}C_2$]acetate was added to each flask, and the incubation was continued for an additional 4 days. The isolation procedure for [1,2-$^{13}C_2$]acetate labeled rumbrin was essentially the same as previously reported[1]. Four mg of [1,2-$^{13}C_2$]acetate labeled rumbrin was obtained from 1 liter culture broth.

References

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