NOVEL NATURAL COLORANTS FROM *MONASCUS ANKA* U-1

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Abstruct———The structure of xanthomonasin A($C_{21}H_{24}O_7$), a natural colorant with a novel carbon skeleton (furanoisophthalide), produced by a mutant strain of *Monascus anka*, has been determined by the application of INADEQUATE experiment as well as by the variety of correlation spectroscopic techniques.

A fungi of the *Monascus* species is a source of a natural colorant which has been used as preservative or red rice wine in China and Taiwan for years. In Japan, a red natural coloring material which is produced by *Monascus anka* has been used as a food additive. During the course of selection of a high yield strain,[1] one mutant strain *M. anka* U-1 was found to produce novel yellow pigments designated as xanthomonasin A and xanthomonasin B. The ethanolic extract(300 ml) of *Monascus anka* U-1 (500 ml culture broth) was purified by repeated chromatography on silica gel using MeOH/$CHCl_3$ and then by hplc to give yellow pigments, xanthomonasin A (430 mg) and xanthomonasin B(290 mg) as oils. Xanthomonasin A showed the $\{M-H\}^-$ peak at m/z 387 in the negative FABms and its molecular formula was determined as $C_{21}H_{24}O_7$ by high resolution positive FABms

m/z: 389.1606({M+H}$^+$), calcd. for $C_{21}H_{25}O_7$: 389.1600. It showed characteristic absorption at 460 nm in MeOH while no $[\alpha]_D$ exhibited. The ^1H and ^{13}C nmr data for xanthomonasin A are collated in the Table 1.

	δ_C(ppm)	*	**	***	****	†	††
A	13.8	Qt	124	3.6	C	a	-
B	17.6	Qdd	127	7.4, 4.6	M	e	-
C	21.9	Tq	124	3.8	F	b	a
D	23.5	Qs	129	-	J	c	-
E	23.9	Tt	126	3.1	H	d	g
F	30.9	Tm	126	-	-	b	a,g
G	36.1	Tbrs	133	-	I	h	-
H	40.7	Tt	127	3.4	U	g	-
I	76.7	Sbrs	-	-	J	-	c,f,h
J	85.3	Sbrs	-	-	I	-	c,h
K	102.6	Sd	-	22	P,T	-	f,h
L	104.7	Ss	-	-	Q,S	-	-
M	125.4	Dq	150	6.8	B	k	e
N	129.3	Sdd	-	6.5, 2.7	Q,P	-	f,h
O	130.8	Dbrs	150	-	I	i	e,f
P	143.2	Sq	-	6.1	G,N,K	-	f,h
Q	153.1	Sd	-	3.1	L,J,N	-	c
R	170.4	Ss	-	-	-	-	-
S	170.5	Ss	-	-	L	-	-
T	182.3	Ds	168	-	K	l	-
U	194.3	Sm	-	-	H,L	-	g

	δ_H(ppm)	
a	0.85	(3H, t, J 7 Hz)
b	1.25	(4H, m)
c	1.42	(3H, s)
d	1.48	(2H, qn., J=7.5 Hz)
e	1.54	(3H, dd, J=6.5, 1.5 Hz)
f	2.76	(1H, d, J=19 Hz)
g	2.77	(2H, t, J=7.5 Hz)
h	3.28	(1H, d, J=19 Hz)
i	5.31	(1H, dq, J=16, 1.5 Hz)
j	5.54	(1H, s)
k	5.59	(1H, dq, J=16, 6.5 Hz)
l	9.42	(1H, s)

Table 1. ^1H and ^{13}C-Nmr (δ/ DMSO-d_6) of xanthomonasin :*Gated decoupled experiments afforded not only signal multiplicities by direct couplings $^1J_{C\text{-}H}$ (capital letters) but fine splitting by long-range couplings $^nJ_{C\text{-}H}(n>1)$ (small letters) which aided speculation about adjacent groups. **$^1J_{C\text{-}H}$, *** $^nJ_{C\text{-}H}(n>1)$(J in Hz).

**** Twenty connectivities were distinguished in the INADEQUATE spectrum for a sample amount of 220 mg obsd at 125.6 MHz; data matrix; 2K X 128, multi-quantum axis was zero filled to 256, delay time adjusted for $J_{C\text{-}C}$=70 Hz; pulse delay 2.2 sec. † Protons attached to the carbons were assigned by shift correlation spectrometry with data matrix; 2K X 256. †† Though carbon proton correlations through long-range couplings $^nJ_{C\text{-}H}(n>1)$ were observed with 60, 80, 100 ms delay time, experiments with delay time for smaller J (eg 100 ms) did not provide any additional correlations beyond those identified with 60 ms.

Signals in the Table 1 are designated alphabetically (δ_H in small and δ_C in capital) in order of chemical shift. First order analysis of the multiplets in ^1H nmr and carbon proton shift correlation spectrum(CH-COSY) of xanthomonasin A indicated the existence of partial structures shown in the Figure 1.

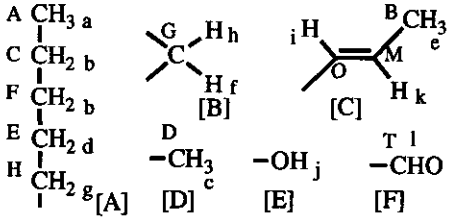

Figure 1. Partial structures deduced by CH-COSY.

These groups, a pentyl (δ_C 13.8, 21.9, 23.9, 30.9, 40.7, δ_H 0.85, 1.25, 1.48, 2.77)[A], a trans-1-propenyl, (δ_C 17.6, 125.4, 130.8, δ_H 1.54,5.31, 5.59)[C], a hydroxyl[E], an aldehyde(δ_C 182.3, δ_H 9.42), methyl (δ_C

23.5, δ_H 1.42)[D], and a methylene[B] groups were suggested to be isolated to one another by proton proton correlation spectra. Whereas xanthomonasin B showed $\{M-H\}^-$ peak at m/z 415 in the negative FABms and its ^1H nmr and ^{13}C nmr revealed the structural similarity to xanthomonasin A except for the longer side chain(C_7H_{11}).

Long-range CH-COSY experiments afforded correlations on 7 quaternary carbons out of 10 unconnected ones (Table 1) to extend the connectivities on the above substructures to as those shown in Figure 2.

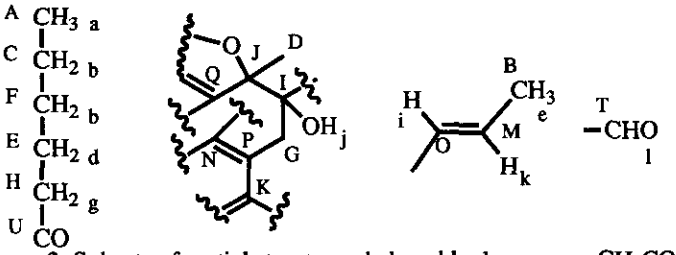

Figure 2. Subsets of partial structures deduced by long range CH-COSY.

The carbons waiting to fill bondings in these partial structures(U, K, N, Q and I) are quaternary ones except O and T. Further atoms adjacent to K, N and Q are again quaternary ones. Left off carbons from partial structures in the Figure 2 are S, L, R in the Table 1 which are also sp^2 quaternary ones.

Since it is impracticable to find out connections through more than two quaternary carbons with carbon-proton shift correlation methods, the INADEQUATE experiments using spin echo[2] provided 6 new connections between U-L, S-L, L-Q, Q-N, O-I and K-T. The resulting structure is consistent with the carbon proton correlations in the Table 1 and the molecular formula by placing the last one atom R adjacent to K as Figure 3. Low signal intensity of R, about one fourth of other signals in the proton noise decoupled ^{13}C nmr, is due to the reason that the R-K pair was buried in the INADEQUATE experiment.

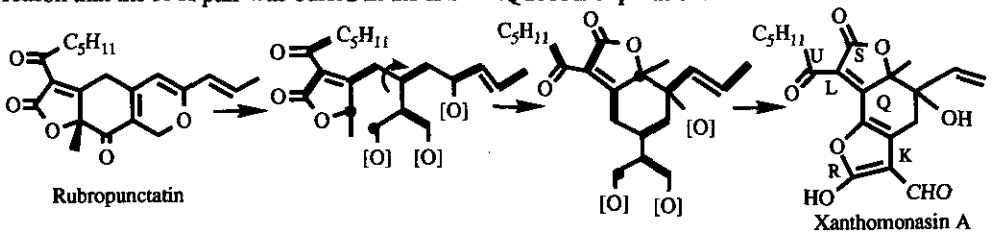

Figure 3. Structure and possible biosynthetic scheme of xanthomonasin A.

Each carbon atom in the structure of Figure 3 is in the correspondingly expected range of chemical shifts. Though the structure of xanthomonasin A in Figure 3 presents a novel skeleton which has never been described

in the *Monascus* family, an oxidative cleavage and a successive rearrangement of rubropunctatin which is one of the major pigments in the family lead easily to the structure of xanthomonasin A.

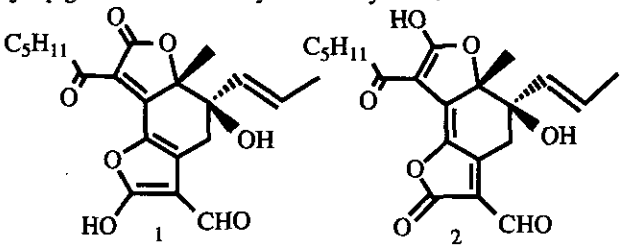

Figure 4. Tautomeric isomer of xanthomonasin A.

The biosynthesis of rubropunctatins was deduced by Hadfield *et al.*[3] To confirm the structure of xanthomonasin A and the above hypothesis, an incorporation experiment of [1,2-$^{13}C_2$] sodium acetate into xanthomonasin A was carried out to give xanthomonasin A (104 mg) 1.1% enriched. The INADEQUATE experiment on the enriched sample demonstrated again 9 C-C coupling pairs of N-Q, K-T, L-S, H-U, I-O, G-P B-M, E-F and A-C due to incorporation of an intact C2 unit as expected from the biogenetical consideration. Though two pairs (M-O and E-H) not due to an intact C2 unit appeared, removal of other 10 pairs from the INADEQUATE spectrum confirmed the structure and the above scheme. Though the specific incorporation ratio of 1.1% is not high, multiple satellites in the proton-noise decoupled ^{13}C nmr revealed simultaneous labelling which caused extra coupling pairs cited above.

The absence of a nOe dipolar relaxation pathway between (D) and (C) observed by phase sensitive NOESY experiment established the stereochemical assignment that the methyl (D) group and the propenyl group(C) are oriented as trans to each other. An ambiguity remains regarding the exact structure because of tautomerism at carbons R and S. The two possible structures are shown in Figure 4. Work is underway to determine the complete structure.

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