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Ceroalbolinic Acid, a Common Body Pigment of Three *Ceroplastes* Scale Insects in Japan. Confirmation of Structure

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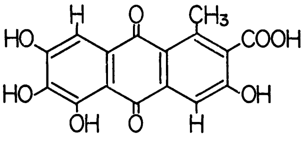
Synopsis. The structure of ceroalbolinic acid, a widely occurring insect pigment, a common body pigment of three Japanese *Ceroplastes* scale insects, has been confirmed as **2** by ^{13}C-NMR long-range selective proton decoupling (LSPD) experiments on its permethylated derivative **3**. Full assignments of skeletal carbons for **3** were also obtained.

The scale insects *Ceroplastes*(Coccidae) are pests which attack persimmon, citrus and other orchard trees and are widely distributed in Japan, Mexico, *etc.* The insect secretion has given rise to numerous new terpenoids[1,2] as well as waxes,[3] whereas one of the body pigments, the red carminic acid,[4] an anthraquinone *C*-glucoside isolated from *Dactylopius coccus*, is popularly used in food coloring and staining.



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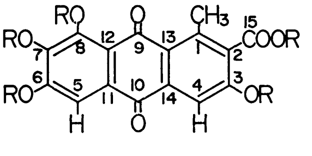

2,R=H; **3**,R=CH$_3$

The three Japanese species of *Ceroplastes*, *C. ceriferus*, *C. floridensis*, and *C. rubens* were treated with chloroform to extract the waxy coating, and then with methanol to extract the orange body pigment. Purification with Sephadex LH-20 and droplet counter-current chromatography(DCCC)[5] gave orange crystals. Yields of the pigment are 0.06, 0.15, and 0.21% respectively from *C. ceriferus*, *C. floridensis*, and *C. rubens* based on their fresh body weights. The ^{1}H-NMR in methanol-d_4 with only three singlets at 7.54, 7.16, and 2.78 ppm in 1:1:3 relative intensities showed the pigment to be identical with ceroalbolinic acid isolated from the Mexican species, *C. albolineatus*.[6—8] Structure **1** was ordiginally proposed,[6] but since a synthetic specimen of structure **1** did not coincide with ceroalbolinic acid, structure **2** was proposed instead without further evidence.[7] Thus, despite the relatively wide occurrence of this pigment, its structure was not settled[9] for long time. Full assignments of ^{13}C-NMR peaks of permethylated derivative **3** by long-range selective proton decoupling(LSPD) led unambiguously to structure **2**.

Permethylation[10] with dimethyl sulfate-potassium carbonate afforded the pentamethyl derivative **3** in quantitative yield. The ^{1}H-NMR peaks of 4-H and 5-H were based on the observation that the 4-H signal was sharpened upon decoupling of the 1-Me singlet. The ^{13}C-gated-^{1}H-decoupled spectrum with NOE show-

Table 1. ^{13}C-NMR T_1 and LSPD experiments; assignments and coupling constants for **3**(in CDCl$_3$)

Carbons	δ	T_1/s	Off resonance	Non decoupled	LSPD of			$^1J_{CH}$	$^2J_{CH}$	$^3J_{CH}$	$^4J_{CH}$
					5-H	4-H	1-Me				
C-9	183.0	16.3	s	bs	bs	bs	*s*				
C-10	182.6	9.6	s	t	*d*	*d*	t			4.4(t)	
C-15	167.7	10.0	s	dq	dq	*q*	dq			4.4(q)	0.8(d)
C-3	158.5	7.6	s	dq	dq	*q*	dq		1.5(d)	4.4(q)	
C-6	156.8	8.5	s	dq	*q*	dp	dq		2.2(d)	4.4(q)	
C-8	154.3	15.4	s	dq	*q*	dq	dq			4.4(q)	0.8(d)
C-7	148.9	12.7	s	dq	*q*	dq	dq			4.4(q),7.3(d)	
C-1	139.9	8.2	s	q	q	q	*s*		5.9(q)		
C-14	136.1	9.1	s	d	d	*s*	d		1.5(d)		
C-2	131.0	13.3	s	dq	dq	*q*	*d*			5.1(q),5.1(d)	
C-11	129.6	10.0	s	d	*s*	d	d		2.2(d)		
C-13	127.4	16.2	s	dq	dq	*q*	*d*			3.7(q),6.6(d)	
C-12	123.2	15.5	s	d	*s*	d	d			6.6(d)	
C-4	106.4	0.6	d	d	d	d	d	166.5(d)			
C-5	105.6	0.6	d	d	d	d	d	165.6(d)			
OMe	61.7	2.4	q	q	q	q	q	146.2(q)			
OMe	61.2	3.7	q	q	q	q	q	146.2(q)			
OMe	56.3	1.3	q	q	q	q	q	145.5(q)			
OMe	56.3	1.3	q	q	q	q	q	145.5(q)			
OMe	52.5	1.7	q	q	q	q	q	148.9(q)			
1-Me	19.2	2.1	q	q	q	q	q	130.5(q)			

Italics denote change in multiplicity. s: Singlet, d: doublet, t: triplet, q: quartet, bs: broad singlet, dq: double quartet.

ed two skeletal carbonyl carbons at 183.0 and 182.6 ppm with broad singlet and triplet(J=4.4 Hz),[11] respectively. Selective irradiation of the 4- and 5-H signals both led to a collapse of the triplet to a doublet, whereas the broad singlet remained unchanged. The 182.6 ppm triplet is thus assigned to C-10 flanked by 4-H and 5-H in structure **3**; if the pentamethyl derivative were represented by the alternative structure, both carbonyls would have been doublets. Relaxation time(T_1) measurement also supported the structure **3**; the T_1 values of the skeletal carbons at lower-half part in the structure **3**, *i.e.* carbons 3,4,5,6, 10,11, and 14, are remarkably shorter compared with those of the upper-half (C-2,7,8,9,12, and 13) because of the presence of two protons in the same side of the ring.

LSPD of 4-H, 5-H, and 1-Me, which were carried out during the course of this study led to assignments of all ^{13}C-NMR peaks, as shown in the table, as well as to a full corroboration of structure **3** for pentamethylated ceroalbolinic acid. The present LSPD method is generally applicable in determining the substitution pattern of polysubstituted aromatic compounds, a problem frequently encountered and difficult to solve otherwise.

Interestingly, similar to the case of carminic acid reported by Eisner *et al.*,[12] ceroalbolinic acid also exhibited antifeedant activity against ants. This ceroalbolinic acid might therefore be working as one of the defense substances together with sticky waxy coating for *Ceroplastes* insects which cannot move anymore once after settled down on a tree, secreting so called honeydews without being attacked by ant or other carnivorous insects.

Experimental

All the melting points are uncorrected. ^{1}H- and ^{13}C-NMR were obtained on a JEOL FX-100 FT instrument at 25 °C, spectral width 2 (^{1}H) and 6 kHz (^{13}C), data points 16 kW, repetition time 5 (^{1}H) and 2.5—3.5 s (^{13}C), pulse width 17 (^{1}H) and 6 μs (^{13}C) (both 45° pulse), power level for LSPD 13 kHz, −50 dB with power attenuator. T_1 experiment was carried out with saturation-recovery method on an undegassed sample. Solvent peak was used as an internal standard for NMR, 7.24 (^{1}H, $CHCl_3$), 3.30 (^{1}H, CHD_2OD), 77.0 (^{13}C, $CDCl_3$), and 49.0 ppm (^{13}C, CD_3OD). EI- and FD-mass spectra were obtained on a JEOL JMS-01SG-2 mass spectrometer.

Isolation of Ceroalbolinic Acid (2). Crude methanol extracts of each insects were separately treated with almost equal volume of chloroform-water mixture (1:1 v/v) to remove lipophilic part. Aqueous layer of the mixture was then concentrated *in vacuo* to a thick syrup followed by column chromatography on a Sephadex LH-20 with 0.1% acetic acid in methanol to give pigment as a retarded band. After further purification on a DCCC (Tokyo Rika Kikai, Co.) with chloroform–methanol-0.02 mol/dm^3 aqueous hydrochloric acid (7:13:8 v/v) using aqueous layer as a mobile phase in ascending mode, ceroalbolinic acid crystallized out in the test tubes on a fraction collector. Mp>250 °C (lit,[6] 290 °C, decomp). FD-MS, m/z 331 ($(M+1)^+$, 100%), ^{13}C-NMR (methanol-d_4): 190.1 (C-9), 182.7 (C-10), 171.4 (C-15), and 20.4 (1-Me).

Methylation of Ceroalbolinic Acid. Ceroalbolinic acid was methylated for 5 h with excess amount of dimethyl sulfate and anhydrous potassium carbonate in refluxing acetone. The reaction was quenched by adding glacial acetic acid. After regular work-up of the mixture, yellow needles of pentamethyl derivative **3** were obtained in quantitative yield upon concentration and trituration with small amount of methanol. Crystals thus obtained were pure enough without further recrystallization. Mp 200.5—201.5 °C (lit,[6] 201—204 °C). ^{1}H-NMR ($CDCl_3$): 7.64 (4-H, br s, $J<1$ Hz), 7.57 (5-H, s), 4.05, 4.02, 4.00 4.00 3.92 (OMe ×5, s's), and 2.69 (1-Me, s). FD-MS, m/z 400 (M^+, 1005), EI-MS, m/z 400 (M^+, 97%), 385 ($(M-CH_3)^+$, 100%), 369 ($(M-OMe)^+$, 23%), and 367 ($(M-33)^+$, 53%).

References

1) Y. Naya, K. Yoshihara, T. Iwashita, H. Komura, K. Nakanishi, and Y. Hata, *J. Am. Chem. Soc.*, **103**, 7009 (1981).

2) L. Quijano, J. S. Calederón, and T. Ríos, *Chem. Lett.*, **1979**, 1387.

3) F. Miyamoto, H. Naoki, Y. Naya, and K. Nakanishi, *Tetrahedron*, **36**, 3481 (1980).

4) The Merck Index, 9th ed., 1844, p 235 (1976); the structure in the Merck Index was revised from an α- to a β-*C*-glucopyranoside: A. Fiecchi, M. Anastasia, G. Galli, and P. Gariboldi, *J. Org. Chem.*, **46**, 1511 (1981). We have also arrived at the same conclusion from ^{1}H-NMR (D_2O) data of carminic acid: 1′-H, 4.95 ppm, d, J=9.8 Hz. We are grateful to San-ei Kagaku Kogyo Co. for a gift of cochineal dye from which carminic acid was isolated.

5) For reviews, see: T. Tanimura, H. Otsuka, and Y. Ogihara, *Kagaku No Ryoiki*, **29**, 895 (1975); K. Hostettmann, *Planta Medica*, **39**, 1 (1980).

6) T. Ríos, *Tetrahedron*, **22**, 1507 (1966).

7) D. D. Gadgil, A. V. Rama Rao, and K. Venkataraman, *Tetrahedron Lett.*, **1968**, 2229.

8) Ceroalbolinic acid has been isolated previously from *C. rubens*: K. Doi, *Sci. Rep. Hirosaki Univ.*, **18**, 37 (1971).

9) Recently, Australian group has managed to synthesize ceroalbolinic acid with correct structure **2**: D. W. Cameron, C. Conn, and G. I. Feutrill, *Aust. J. Chem.*, **34**, 1945 (1981).

10) When smaller amount of dimethyl sulfate (*ca.* 8 mol equiv.) was used, less polar derivative, methyl ceroalbolinate 3,6,7-trimethyl ether, was obtained in addition to **3**. ^{1}H-NMR ($CDCl_3$): 13.12 (8-OH, s), 7.73 (4-H, br s), 7.39 (5-H, s), 4.03, 4.02, 4.02, 3.97 (OMe×4, s's), and 2.73 (1-Me, s). EI-MS, m/z 386 (M^+, 100%), 371 ($(M-CH_3)^+$, 50%), 355 ($(M-OMe)^+$, 21%), and 353 ($(M-33)^+$, 21%).

11) Höfle reported in his extensive studies on ^{13}C-NMR of some naphtho- and anthraquinones, a carbonyl carbon flanked with two protons at *peri*-positions appeared as triplet with $^3J_{CH}$=4.5 Hz for a simple anthraquinone derivative: G. Höfle, *Tetrahedron*, **33**, 1963 (1977).

12) T. Eisner, S. Nowicki, M. Goetz, and J. Meinwald, *Science*, **208**, 1039 (1980).