of α-cyanobenzylidene-*p*-dimethylaminoaniline (Iw) (Fig. 1) is closely related to that of *p*-nitrobenzylidene-*p*-dimethylaminoaniline which shows strong absorption at 275 and 440 nm in acetonitrile,[5] indicating that Iw has an almost coplanar structure.

The 403-nm band of Ix can be regarded as band II because the spectrum of Ix (Fig. 1) is similar to that of *p*-dimethylaminobenzylideneaniline in which the 262-nm band of II exhibits a large red shift owing to the strong electron-donating effect of the dimethylamino group and eventually becomes the longest wavelength band.[2g] Band I of Ix can be expected around the same wave length as that of Ia, but may be hidden by the strong absorption of band II.

Experimental

α-Cyanobenzylideneanilines, Ia—v and x, were prepared from the corresponding nitrones by the method of Krönke and Krönke,[7] and Iw was prepared from *p*-nitrosodimethylaminoaniline and benzylcyanide by the method of Ehrlich and Sachs.[8] All compounds gave expected analytical results.

Spectral data were obtained using Hitachi type 124 and type 100-40 spectrometers.

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Studies on *Cerbera*. II.[1] Cerbinal and Its Derivatives, Yellow Pigments in the Bark of *Cerbera manghas* L.

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Cerbinal, yellow pigment having iridoid skeletal with $\Delta^{3,5,7,9(1)}$-tetraene, and 10-formyl and 11-carbomethoxy residues, was isolated along with 10-carboxy- (cerberic acid) and 11-carboxy- (cerberinic acid) derivatives from the bark of *Cerbera manghas* L.

Keywords——*Cerbera manghas*; Apocynaceae; iridoids; iridoid pigments; theviridoside

In the preceding paper, we reported on the cardenolides in the kernels,[1,3] bark,[1] and leaves[1] of *Cerbera manghas*. On the iridoid constituents of this plant, theviridoside and theveside have been isolated from the leaves by Inouye, *et al.*[4] This paper deals with the isolation and structure elucidation of cerbinal (CL), cerberic acid (CA), and cerberinic acid (CNA), yellow pigments with iridoid skeletal, in the stem and root barks.

When the methanol percolate of the dried powdered bark was concentrated, diluted with water and partitioned with a variety of solvents, CL was isolated as yellow needles by the crystallization with methanol of the benzene fraction. On column chromatography of

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2) Location: *Nanakuma 11, Nishi-ku, Fukuoka*; *a*) To whom, all inquiries should be addressed.
3) *a*) T. Matsubara, *Nippon Kagakukaishi*, **58**, 1104 (1939); *b*) S. Rangaswami and E. Venkata Rao, *J. Sci. Ind. Res.* (India), **16B**, 209 (1957); E. Venkata Rao, B.S. Sastry, and M.N. Narayanan, *Ind. J. Pharm.*, **36**, 75 (1974); E. Venkata Rao and M. Appa Rao, *Phytochemistry*, **15**, 848 (1976).
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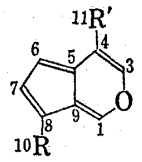
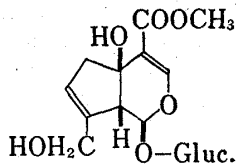
the mother liquor portion of CL, two minor pigments, CA and CNA, were obtained along with CL.





cerbinal:	R=CHO R'=$COOCH_3$	theviridoside
cerberic acid:	R=COOH R'=$COOCH_3$	
cerberinic acid:	R=CHO R'=COOH	
baldrinal:	R=CHO R'=CH_2OAc	

CL exhibited absorption maxima at 254, 282, 292, 333, and 430 nm, and mass spectrum (MS) peaks at *m/e* 203, 175, 145, together with molecular peak at 204. Nuclear magnetic resonance (NMR) indicated 3H singlet at δ 3.99, 1H singlet at 9.90 ppm, and four olefinic protons at 7.11 (d, 4Hz), 7.91 (d, 4 Hz), 8.46 (s), and 9.12 (s) ppm. CL appeared to have a carbomethoxy group by MS peak at 145 ($M^+ - COOCH_3$) and resonance at δ 3.99, and presence of a formyl group was confirmed by the formation of phenylhydrazone, as well as by the appearance of M^+-1 peak, and 1H signal at δ 9.90 ppm on NMR spectrum. Molecular formula of CL, $C_{11}H_8O_4$, suggested that this substance belonged to monoterpenoid with three or four olefinic linkages in tri- or bi-cyclic system, respectively, beside two carbonyl groups. As a structure consistent with these conditions, iridoid framework, in which the formyl or carbomethoxy group conjugated to polyene system, was considered to be most likely. In 1968, baldrinal[5] was reported as an iridoid pigment, having a formyl residue conjugated to four double bonds. Its absorption band (λ_{max} 227, 244, 287, 435 nm) and NMR spectrum indicated quite approximate pattern with those of CL, except for long range coupling between C_1 and C_6 protons, and the resonance due to the protons attached to C_{11} in baldrinal. Therefore, CL was assumed to be either C_{11}-carbomethoxy derivative of baldrinal or its C_{11}-formyl-C_{10}-carbomethoxy isomer, in which the former seemed more preferable because theviridoside, as same as many natural iridoids, has C_{11}-carbomethoxy residue. Although dehydration of C_1–OH to $\Delta^{1(9)}$ has not been known, CL was furnished as a minor product along with unidentified many substances, when theviridoside was treated with Jones' reagent in anticipation of the oxidation of C_{10}–OH and the formation of the conjugated tetraene system, and thus the structure of CL was established.

CA also indicated absorption maxima at 238, 260 (sh), 272, 282, 330, and 425 nm, and NMR spectrum showed 3H singlet for carbomethoxy, and four olefinic protons at 7.22, 8.24, 8.42, and 9.23 ppm, but no resonance corresponding to 9.90 ppm in CL. CA therefore was assumed to be an oxidized derivative of the formyl group of CL. The structure was finally elucidated by the oxidation of CL with CrO_3 in acetic acid to afford CA.

CNA was obtained as solid. Its absorptions in ultraviolet and visible ranges, λ_{max} 250, 293, 310 (sh), and 433 nm were close to those of CL and CA. On NMR, however, no carbomethoxy protons were found, whereas five protons ascribable to formyl and olefinic groups were observed. CNA was revealed to be demethyl derivative of CL, having been converted into CL by methylation with CH_2N_2.

CL, CA, and CNA were isolated from fifteen to twenty years old tree, but not from the leaves or bark of young plants, which contain a large amount of theviridoside, and seem to be formed in aged or mortal tissue of the bark. It is therefore unlikely to consider that the pigments are converted from the iridoid glucosides during a process of collection, extraction or isolation as in the case of baldrinal.

Experimental

Melting points were measured on Kofler block and uncorrected. MS, NMR, and infrared (IR) spectra were run using JEOL JMS-OISG mass spectrometer, Hitachi R-22 spectrometer, and EPI-G3 IR spectrometer, respectively.

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Extraction and Isolation of the Pigments——The barks (4.0 kg of stem bark, 1.9 kg of root bark) were collected in Feb. 1974, in Kunigami-Son, Okinawa, dried in the shade and powdered. After percolation with MeOH, the percolates were concentrated respectively and diluted with water, keeping MeOH concentration as 50%. The mixture was defatted with hexane and then extracted with benzene. The benzene extractives (23.5 g from stem bark, and 6.2 g from root bark) were dissolved in MeOH and allowed to stand overnight. The yellow needles (CL) were filtered (120 mg from s.b. and 300 mg from r.b.), and the mother liquor was combined together and subjected to column chromatography on silica gel with benzene–acetone as eluant to afford 130 mg of CL, 50 mg of CA, and 40 mg of CNA, prior to the elution of the cardenolides, cerberin and neriifolin.

Cerbinal (CL)——Long needles were obtained by recrystallization from MeOH, mp 188—189°, λ_{max}^{MeOH} nm 254, 282, 292, 333, 430. *Anal.* Calcd. for $C_{11}H_8O_4$: C, 64.70; H, 3.95. Found: C, 64.68; H, 3.91. NMR (in $CDCl_3$) δ (ppm): 3.99 (3H, s, $-COOCH_3$), 7.11 and 7.91 (1H each, d, $J=4$ Hz, C-6 and -7), 8.46 and 9.12 1H each, s, C-1 and -3), 9.90 (1H, s, –CHO). MS *m/e*: 204.0437 (M^+; Calcd. for $C_{11}H_8O_4$: 204.0422), 203 (M^+-1), 175 (M^+-CHO), 173 (M^+-OCH_3), 145 ($M^+-COOCH_3$).

CL (120 mg) was dissolved in EtOH and heated on water bath for 30 min with phenylhydrazine·HCl (80 mg) and AcONa (120 mg). The phenylhydrazone was crystallized from benzene–acetone to give needles, mp 197—210° (dec.).

Theviridoside (700 mg) was oxidized for 30 min at room temp. with Jones' reagent. The reaction mixture was diluted with water and extracted with BuOH. BuOH ext. was subjected to column chromatography and then to preparative thin-layer chromatography (TLC), and 10 mg of CL was obtained as needles after crystallization from MeOH, mp 187—189°. On admixture of the sample with authentic CL, no melting point depression was observed and IR spectra of the both were superimposable.

Cerberic Acid (CA)——CA was crystallized from acetone to give needles, mp 213—223° (dec.), λ_{max}^{MeOH}: 238, 260 (sh), 272, 282, 330, 425. NMR: 3.84 (3H, s, $-COOCH_3$), 7.22 and 8.24 (1H each, d, $J=4$ Hz, C-6 and -7), 8.42 and 9.23 (1H each, s, C-1 and -3). CL (250 mg) was dissolved in AcOH (30 ml) and stirred with CrO_3 (120 mg) in AcOH (2.5 ml) and H_2O (0.5 ml) for 1.5 hr at room temp. The mixture was extracted with $CHCl_3$ and passed through silica gel column. CA (20 mg) was obtained as needles after crystallization from acetone, mp 210—224°. IR was coincided with that of CA obtained from the bark.

Cerberinic Acid (CNA)——λ_{max}^{MeOH}: 250, 293, 310 (sh), 433. NMR: 7.20 and 8.00 (1H each, d, $J=4$ Hz, C-6 and C-7), 8.48 and 9.20 (1H each, s, C-1 and -3), 9.78 (1H, s, -CHO). CNA was treated with CH_2N_2 in ether and the product was crystallized from hexane–AcOEt to give needles, mp 183—188°. IR was in good agreement with that of CL from the bark.

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