UDC 547.976 : 582.972

73. Yoshio Hirose : Syntheses of Damnacanthal, Damnacanthol, Norjuzunal, and Norjuzunol, the Coloring Matters of *Damnacanthus* Spp.*2

(*Pharmaceutical Faculty, University of Kumamoto*[*1])

Nonomura,[1] and Nonomura and Hirose[2] reported previously that *Damnacanthus major* SIEB. ET ZUCC., *D. major* SIEB. ET ZUCC. VAR. *parvifolius* KOIDZ., and *D. indicus* GAERTNER FIL. VAR. *microphyllus* MAKINO contain anthraquinone pigments named damnacanthal, damnacanthol, and juzunal, for which the structural formulae, 1-methoxy-2-formyl-3-hydroxy- (I), 1-methoxy-2-hydroxymethyl-3-hydroxy- (II), and 1-methoxy-2-formyl-3,x-dihydroxy-anthraquinone (III) were proposed, respectively.

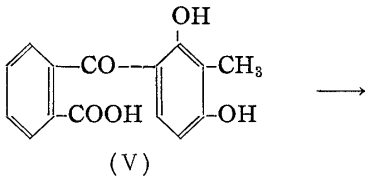
The present paper deals with the syntheses[3] of damnacanthal, damnacanthol, and norjuzunal, a demethylated product of natural juzunal.

A homolog of juzunal was newly isolated from the above *Damnacanthus* spp., which was designated juzunol (IV). The synthesis of juzunol was also achieved.

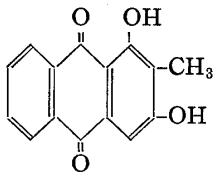
As the starting material for the synthesis of damnacanthol and damnacanthal, rubiadin (VI) was employed, which was prepared by the cyclization of 2,4-dihydroxy-3-methylbenzophenone-2′-carboxylic acid (V), m.p. 223°, with conc. sulfuric acid and boric acid.

(I) R=CHO
(II) R=CH_2OH

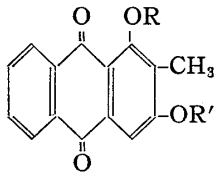
(III) R=CHO
(IV) R=CH_2OH



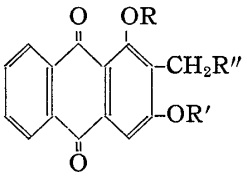
(V)



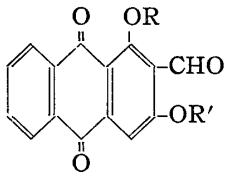
(VI)



(VII) R=H, R′=$COCH_3$
(VIII) R=CH_3, R′=$COCH_3$



(IX) R=CH_3, R′=$COCH_3$, R″=Br
(X) R=CH_3, R′=$COCH_3$, R″=$OCOCH_3$
(XI) R=CH_3, R′=H, R″=OH



(XII) R=CH_3, R′=H

*1 Oe-Machi, Kumamoto (広瀬良男).

*2 After submitting this manuscript, the author learned that N. R. Ayyanger, B. S. Joshi, and K. Venkataraman (Tetrahedron, **6**, 331(1959)) reported the synthesis of damnacanthol and damnacanthal.

1) S. Nonomura : Yakugaku Zasshi, **75**, 219, 222, 225(1955).
2) S. Nonomura, Y. Hirose : *Ibid.*, **75**, 1305(1955).
3) Preliminary reports cf. Y. Hirose : *Ibid.*, **76**, 1148(1956); **78**, 947(1958).

1-O-Methyl-3-O-acetylrubiadin[4](Ⅷ) was prepared by partial acetylation of rubiadin with boroacetic anhydride in acetic anhydride followed by methylation with methyl iodide and silver oxide.

Bromination of (Ⅷ) with Wohl-Ziegler's reagent gave 1-methoxy-2-bromomethyl-3-acetoxyanthraquinone (Ⅸ), m.p. 169~170°, which was readily converted into 1-methoxy-2-acetoxymethyl-3-acetoxyanthraquinone (X), m.p. 156~157°, by heating with sodium acetate and acetic anhydride. The product (X) was proved to be identical with the acetate of natural damnacanthol, m.p. 157°, by a mixed fusion, and the deacetylated product, 1-methoxy-2-hydroxymethyl-3-hydroxyanthraquinone (XI), m.p. >300°(decomp.), was identified with damnacanthol by the physical properties and infrared spectrum.

Oxidation of synthetical damnacanthol with active manganese dioxide in ethyl acetate afforded 1-methoxy-2-formyl-3-hydroxyanthraquinone (Ⅻ), m.p. 211~212°, which was established by a mixed fusion to be identical with natural damnacanthal, m.p. 211~212°. The melting point of damnacanthal recorded in the previous report (208°) should be corrected to that given by the highly purified specimen. The identity of synthetic and natural damnacanthal was confirmed by comparison of their monoacetate, m.p. 191~191.5°, demethylated product, m.p. 220~221°, and anil, m.p. 232~232.5°.

Thus the structures of damnacanthol (Ⅱ) and damnacanthal (I) were fully established.

In the previous work, juzunal was suggested to be hydroxy-damnacanthal while there was not provided any definite proof for the structure, and, in particular, the position of one of the hydroxyls remained unsettled.

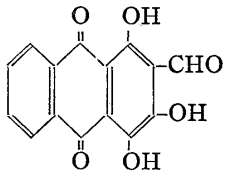
Juzunal exhibits infrared absorption maxima at 1648, 1626, 1582, and 1548 cm^{-1}, which were assigned to non-chelated quinone C=O band overlapped with chelated aromatic aldehyde C=O band, chelated quinone C=O, and phenyl double bonds, respectively.

The infrared spectrum of norjuzunal showed the presence of chelated aromatic aldehyde (1638cm^{-1}), chelated quinone C=O (1616 cm^{-1}), and the absence of non-chelated quinone C=O.

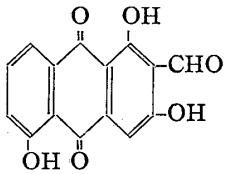
The absorption band of phenyl at 1582 cm^{-1} (SS)*[3] shown in the infrared spectrum of norjuzunal suggested the presence of hydroxyls in the 1,3,4-*[4] or 1,3,5-positions*[5] in anthraquinone ring.[5]

As a reference of the absorption of aldehydic C=O in juzunal and norjuzunal, the infrared spectra of 2,6-dihydroxy- and 2-methoxy-6-hydroxybenzaldehyde were measured. The former compound gave a hydroxyl band at 3572 cm^{-1} and a chelated C=O band at 1635 cm^{-1} along with phenyl bands at 1617 and 1582 cm^{-1}. The latter compound showed the absence of free hydroxyl band above 3000 cm^{-1}, and the presence of chelated C=O at 1638 cm^{-1} and phenyl bands at 1614 and 1582 cm^{-1}.

On the basis of infrared spectral analysis, two alternative formulae, 1,3,4-trihydroxy-(Ⅻ) and 1,3,5-trihydroxy-2-formylanthraquinone (XIV) were considered to represent norjuzunal.



(XIII)



(XIV)

*3 SSS and S marks show qualitatively that the intensity of phenyl band absorption is stronger than and the same as $\nu_{C=O}$ intensity, respectively. SS mark shows intermediate grade between SSS and S.

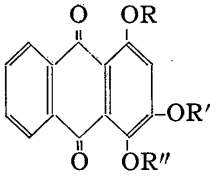
*4 IR ν_{max}^{Nujol} cm^{-1} : 3330 (-OH), 1617 (chelated C=O), 1582 (phenyl (SSS)). O. Tanaka : Abstr. of Papers, 77th Annual Meeting, Pharmaceutical Society of Japan, 259(1957).

*5 IR ν_{max}^{Nujol} cm^{-1} : 3300 (-OH), 1597 (chelated C=O), 1582 (S) (phenyl) (authentic sample).

4) E. T. Jones, A. Robertson : J. Chem. Soc., **1930**, 1699.

5) O. Tanaka : This Bulletin, **6**, 18, 24(1958).

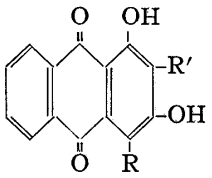
The synthesis of 1,3,4-trihydroxy-2-formylanthraquinone (XIII) was first attempted starting with hydrolysis of 1,3-di-O-acetyl-4-O-methylpurpurin (XV), m.p. 202~204°, with methanolic sulfuric acid or with 10% caustic alkali in acetone solution where the expected 4-O-methylpurpurin (XVI) was not obtained, but 3-O-methylpurpurin (XVII) was formed by the rearrangement of O-methyl group.



(XV) $R=R'=COCH_3$, $R''=CH_3$
(XVI) $R=R'=H$, $R''=CH_3$
(XVII) $R=R''=H$, $R'=CH_3$
(XVIII) $R=R''=COCH_3$, $R'=CH_3$

This arrangement of methyl was confirmed by acetylation of the product giving 1,4-diacetoxy-3-methoxyanthraquinone (XVIII), m.p. 170~172°.[6] The hydroxymethylation of 3-O-methylpurpurin (XVII) failed.

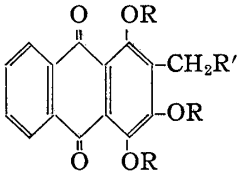
Subsequently, 4-bromorubiadin[7] (XIX) was employed as the starting material, which was converted into 2-methylpurpurin (XX), m.p. 266~267°, by the action of conc. sulfuric acid and fused boric acid.



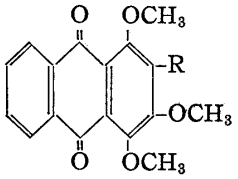
(XIX) $R=Br$, $R'=CH_3$
(XX) $R=OH$, $R'=CH_3$

Triacetate of 2-methylpurpurin (XXI), m.p. 213°, was treated with N-bromosuccinimide in carbon tetrachloride to give 1,3,4-tri-O-acetyl-2-bromomethylpurpurin (XXII), m.p. 219~220°, which was converted into tri-O-acetyl-2-acetoxymethylpurpurin (XXIII), m.p. 182~183°.

On the other hand, 2-methylpurpurin trimethyl ether (XXIV), m.p. 126~127°, was used as the starting material, which was brominated and followed by several steps of reactions to give 1,3,4-tri-O-methyl-2-formylpurpurin (XXVIII), m.p. 136~137°.



(XXII) $R=COCH_3$, $R'=Br$
(XXIII) $R=COCH_3$, $R'=OCOCH_3$



(XXIV) $R=CH_3$ (XXV) $R=CH_2Br$
(XXVI) $R=CH_2OCOCH_3$
(XXVII) $R=CH_2OH$ (XXVIII) $R=CHO$

The melting points of the purpurin-type product (XXVIII) and its derivatives synthesized as above showed no agreement with those of natural juzunal and its corresponding derivatives.

Hence, 1,3,5-trihydroxy-2-formylanthraquinone (XIV) appeared as the most possible structure for norjuzunal.

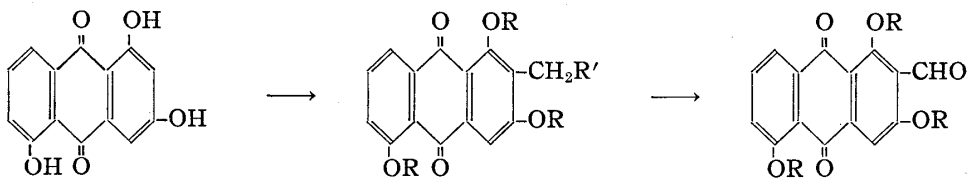
The method of introducing hydroxymethyl grouping which was used for the syntheses of lucidin[8] and 3,6-dihydroxymethylanthrachryson[9] was adopted for 1,3,5-trihydroxyanthraquinone (XXIX) to obtain 1,3,5-trihydroxy-2-hydroxymethylanthraquinone

6) A. G. Perkin, R. C. Storey : J. Chem. Soc., **1928**, 231.
7) O. Tanaka : This Bulletin, **6**, 203(1958).
8) N. R. Ayyangar, K. Venkataraman : J. Sci. Ind. Res. India, **15B**, 359(1956).
9) DRP 184,786 (Frdl., **IX**, 694).

(XXX), m.p. >300°, which was proved to be identical with norjuzunol derived from natural juzunol (IV). A mixed fusion of tetraacetate of synthetic 1,3,5-trihydroxy-2-hydroxymethylanthraquinone, m.p. 236~237°, with tetra-O-acetyl-norjuzunol showed no depression of melting point.

Finally, under the same condition of oxidation of damnacanthol into damnacanthal, 1,3,5-trihydroxy-2-hydroxymethylanthraquinone was converted to 1,3,5-trihydroxy-2-formylanthraquinone (XIV, XXXII), m.p. 266~267°, which was confirmed by a mixed fusion and by its infrared spectrum to be identical with norjuzunal derived from natural juzunal.

Since it was observed that juzunal and juzunol were demethylated readily by boiling with 48% hydrobromic acid in glacial acetic acid, the methoxyl group seemed to be present in the α-position of the anthraquinone ring.



(XXIX) (XXX) R=H, R'=OH (XXXI) R=$COCH_3$, R'=$OCOCH_3$ (XXXII) R=H

Referring to the fact that juzunal and juzunol are coexisting with damnacanthal (I) and damnacanthol (II) in the same plant, and from biochemical point of view, it seems most likely that the methoxyl in juzunal and juzunol occupies the corresponding position of that in damnacanthal and damnacanthol.

Thus the structures of juzunal and juzunol have finally been established as represented by the formulae (XXXIII) and (XXXIV), respectively.

(XXXIII) (XXXIV)

As observed in protocetraric acid,[10] the carbinol group in juzunol (XXXIV) and damnacanthol (II) was readily alkylated when heated with butanol to form 2-O-butyl derivative.

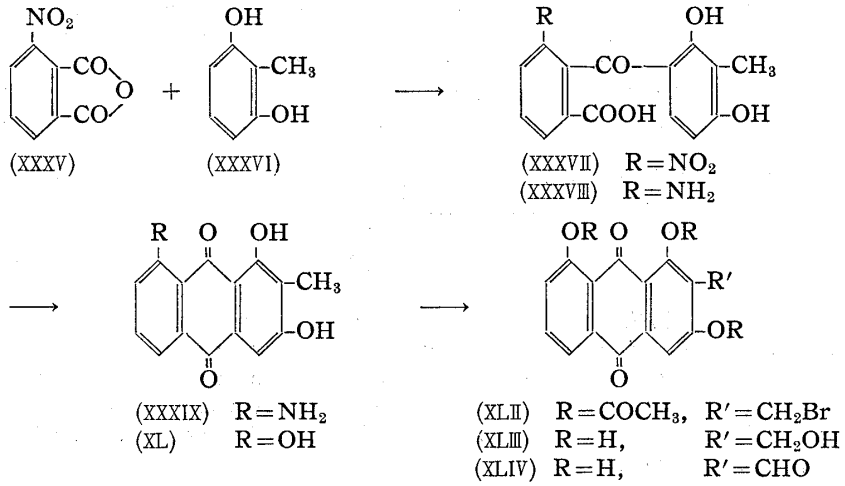
In connection with synthetical study of juzunal, preparation of 1,3,8-trihydroxy-2-formylanthraquinone was undertaken.

3-Nitrophthalic anhydride (XXXV) was condensed with 2,6-dihydroxytoluene (XXXVI) to give 2,4-dihydroxy-3-methyl-6'-nitrobenzophenone-2'-carboxylic acid (XXXVII), m.p. 252°, which was converted into the corresponding amino derivative (XXXVIII), m.p. 230°, by catalytic reaction. The ring closure of the compound (XXXVIII) with sulfuric acid afforded 1,3-dihydroxy-2-methyl-8-aminoanthraquinone (XXXIX), m.p. 354°. On diazotization of the amino-anthraquinone derivative, 1,3,8-trihydroxy-2-methylanthraquinone (XL), m.p. 312°, was formed, whose triacetate was treated with N-bromosuccinimide and benzoyl peroxide in carbon terachloride to give 1,3,8-triacetoxy-2-bromomethylanthraquinone (XLII), m.p. 222°. On treatment with acetic anhydride and fused sodium acetate followed by cautious hydrolysis, the compound (XLII) yielded 1,3,8-trihydroxy-2-hydroxymethylanthraquinone (XLIII), m.p. 282°(decomp.), which was converted into 1,3,8-trihydroxy-2-formylanthra-

10) Y. Asahina, Y. Tanase: Ber., **67**, 766(1934).

quinone (XLIV), m.p. 216°. The synthesized aldehydic anthraquinone (XLIV) showed the infrared absorption bands at 1655 cm^{-1} for free quinone C=O band overlaping with the aromatic aldehyde C=O chelated with the neighboring OH group, and at 1619 cm^{-1} for the chelated quinone C=O grouping. The absorption band of phenyl of 1,3,8-trisubstituted anthraquinone appeared at 1555~1592 cm^{-1}(broad shoulder).

1,3,8-Trihydroxy-2-hydroxymethylanthraquinone (XLIII) was also prepared by hydroxymethylation of 1,3,8-trihydroxyanthraquinone.[11]



(XXXV) (XXXVI) (XXXVII) R=NO_2; (XXXVIII) R=NH_2; (XXXIX) R=NH_2; (XL) R=OH; (XLII) R=$COCH_3$, R′=CH_2Br; (XLIII) R=H, R′=CH_2OH; (XLIV) R=H, R′=CHO

This indicated that the Friedel-Crafts condensation of 3-nitrophthalic anhydride (XXXV) and 2,6-dihydroxytoluene (XXXVI) gave (XXXVII) as the sole product, which was converted into (XLIII) by several steps as described above.

Much differences have been observed in the properties and infrared spectra of 1,3,8-trihydroxy-2-formylanthraquinone and norjuzunal. It is noted that juzunal and juzunol are the first examples of naturally occurring 1,3,5-trihydroxyanthraquinone derivatives.

Experimental

2,4-Dihydroxy-3-methylbenzophenone-2′-carboxylic Acid (V)—To a solution of phthalic anhydride (0.6 g.) and 2,6-dihydroxytoluene (0.6 g.) in $CHCl_2CHCl_2$ (50 cc.), $AlCl_3$ (1.2 g.) was added under stirring. The mixture was heated at 125° for 3 hr., 10% HCl (50 cc.) was added to the cooled reaction mixture, and $CHCl_2CHCl_2$ and unchanged phenol were removed by steam distillation. The residue was dissolved in 5% $NaHCO_3$, filtered, and the filtrate was acidified with 10% HCl. The product was crystallized from benzene and then from AcOH to colorless rhombic plates (yield, 0.4 g., 33%), m.p. 223°. *Anal.* Calcd. for $C_{15}H_{12}O_5$: C, 66.17; H, 4.44. Found: C, 66.19; H, 4.58.

Rubiadin (VI)—A solution of the acid (V) (0.4 g.) and fused H_3BO_3 (0.8 g.) in conc. H_2SO_4 (5 cc.) was heated on a boiling water bath for 25 min. When cool, the reaction mixture was poured on ice The precipitate, on recrystallization from benzene, gave (VI) as yellow needles, m.p. 290° (yield, 0.2 g., 54%), which was proved by mixed fusion to be identical with rubiadin prepared by condensation of BzOH and 3,5-dihydroxy-*p*-toluic acid according to the method of Kusaka.[12] *Anal.* Calcd. for $C_{15}H_{10}O_4$: C, 70.86; H, 3.96. Found: C, 70.92; H, 4.08.

1-Hydroxy-3-acetoxy-2-methylanthraquinone (VII) and 1-Methoxy-3-acetoxy-2-methylanthraquinone (VIII)—These compounds were synthesized according to the method of Robertson.[4]

1-Methoxy-2-bromomethyl-3-acetoxyanthraquinone (IX)—A solution of (VIII) (0.5 g.), N-bromosuccinimide (0.5 g.), and BzOOH (0.05 g.) in dried CCl_4 (50 cc.) was refluxed for 12 hr. The solvent was distilled off, the viscous residue was washed with hot water, dried, and recrystallized from EtOH to yellow needles, m.p. 169~170°; yield, 0.4 g. *Anal.* Calcd. for $C_{18}H_{13}O_5Br$: C, 55.52; H, 3.34. Found: C, 55.52; H, 3.37.

1-Methoxy-2-acetoxymethyl-3-acetoxyanthraquinone (X)—A solution of (IX) (0.4 g.), fused NaOAc

11) W. K. Anslow, J. Breen, H. Raistrick: J. Chem. Soc., **1940**, 427
12) T. Kusaka: Yakugaku Zasshi, **55**, 682(1935).

(0.4 g.), and Ac_2O (15 cc.) was refluxed for 1 hr. The reaction mixture was poured on crushed ice. The precipitate was separated and recrystallized from EtOH to pale yellow needles (0.3 g.), m.p. 156~157°, alone and on admixture with the natural diacetyl-damnacanthol. *Anal.* Calcd. for $C_{20}H_{16}O_7$: C, 65.21; H, 4.38. Found: C, 65.25; H, 4.40.

1-Methoxy-2-hydroxymethyl-3-hydroxyanthraquinone (Damnacanthol) (XI)—To a solution of (X) (0.5 g.) in MeOH (75 cc.), conc. H_2SO_4 (1.5 cc.) was added and refluxed for 0.5 hr. After dilution with water the precipitate was recrystallized from acetone to obtain pale yellow needles (0.2 g.), m.p. >300°(decomp., darkens gradually and gives no definite melting point owing to its sensibility to alkaline contamination in the capillary glass). It gives a brown coloration with $FeCl_3$ in EtOH solution, a yellow orange coloration with $Mg(AcO)_2$ in MeOH, and a red coloration with BuOH saturated with 27% NH_4OH, by which it was developed on paper chromatogram giving a spot of Rf. 0.55. The properties of this synthetic product showed an agreement with those of natural damnacanthol. The infrared spectra of these two specimens were entirely identical. *Anal.* Calcd. for $C_{16}H_{12}O_6$: C, 67.60; H, 4.26. Found: C, 66.95, 68.17; H, 4.50, 4.42. IR ν_{max}^{Nujol} cm^{-1}: 3272 (OH), 1662 and 1638 (non-chelated CO), 1575, 1555 (phenyl bands).

Damnacanthol 2-Ethyl Ether—(IX) (0.1 g.) was added to a solution of 99.8% EtOH (30 cc.) and fused NaOAc (0.1 g.). The whole was refluxed for 1 hr. EtOH was distilled off, the residue was extracted with 2% NaOH, and filtered. After acidification of the filtrate the precipitate was recrystallized from acetone to yellow needles (50 mg.), m.p. 198~199°. *Anal.* Calcd. for $C_{18}H_{16}O_5$: C, 69.22; H, 5.16. Found: C, 69.03; H, 4.98.

Damnacanthol 3-Methyl Ether—A solution of (XI) (0.1 g.) in acetone (50 cc.) was heated with Me_2SO_4 (0.5 g.) and anhyd. K_2CO_3 (2 g.) for 3 hr. After filtration the solution was evaporated, the residue was added with 5% NaOH, and the resulting precipitate was crystallized from EtOH to pale yellow needles (80 mg.), m.p. 175°, which was proved to be identical with damnacanthol monomethyl ether by admixture. *Anal.* Calcd. for $C_{17}H_{14}O_5$: C, 68.45; H, 4.73. Found: C, 68.39; H, 4.56. IR ν_{max}^{Nujol} cm^{-1}: 3280 (OH), 1670 (non-chelated CO).

Damnacanthol 2,3-Dimethyl Ether—The reaction mixture mentioned above was refluxed for 12 hr. The product obtained was crystallized from EtOH to give pale yellow needles, m.p. 161~162°. *Anal.* Calcd. for $C_{18}H_{16}O_5$: C, 69.20; H, 5.10. Found: C, 69.42; H, 5.36.

1-Methoxy-2-formyl-3-hydroxyanthraquinone (Damnacanthal) (XII)—A mixture of synthetic damnacanthol (XI) (0.2 g.) and active MnO_2 (1 g.) in AcOEt (50 cc.) was stirred for 3 hr. at a room temperature. The filtrate and acetone extract of the reaction precipitate were distilled off. The product obtained was recrystallized from acetone or AcOEt to yellow needles, m.p. 211~212°, which showed no depression on admixture with the natural damnacanthal, m.p. 211~212°. The infrared spectra of these two specimens were entirely identical. *Anal.* Calcd. for $C_{16}H_{10}O_5$: C, 68.08; H, 3.57. Found: C, 68.32; H, 3.60. IR ν_{max}^{Nujol} cm^{-1}: 1662 (non-chelated CO), 1634 (chelated aromatic aldehyde), 1575 and 1555 (phenyl bands).

This product is soluble in 5% $NaHCO_3$ giving an orange solution. It dissolves in 10% NaOH with an orange color changing to violet via red color. It gives a brown coloration with $FeCl_3$ in EtOH, an orange-red coloration with $Mg(AcO)_2$, a brown red coloration with NH_4OH-saturated BuOH, and gives a spot at Rf 0.66 on paper chromatogram.

1-Methoxy-2-formyl-3-hydroxyanthraquinone Anilide—m.p. 232~232.5°; mixed m.p. 232~232.5° with that prepared from natural damnacanthal. *Anal.* Calcd. for $C_{22}H_{15}O_4N$: C, 73.94; H, 4.23. Found: C, 73.85; H, 4.24.

1,3-Dihydroxy-2-formylanthraquinone (Nor-damnacanthal)—m.p. 220~221°, mixed m.p. 220~221° with nor-damnacanthal. *Anal.* Calcd. for $C_{15}H_8O_5$: C, 67.17; H, 3.01. Found: C, 67.24; H, 3.39.

1-Methoxy-2-formyl-3-acetoxyanthraquinone (O-Acetyldamnacanthal)—m.p. 191~191.5°, mixed m.p. 191~191.5° with O-acetyldamnacanthal. *Anal.* Calcd. for $C_{18}H_{12}O_6$: C, 66.67; H, 3.37. Found: C, 66.75; H, 4.03.

Isolation of the Components of Damnacanthus plants—The roots of *Damnacanthus major* Sieb. et Zucc. (1 kg.) were extracted thoroughly with acetone. The acetone extract (10 g.) was dissolved in the same solvent by heating with activated carbon and concentrated after filtration. The separated precipitate was collected by suction, washed with hydr. acetone to exclude resin, and dried. The collected mass was dissolved again in acetone (300 cc.), added with a small amount of activated carbon, and concentrated to 70 cc. after filtration. The crystalline substance which separated out on standing overnight was repeatedly recrystallized from acetone to give juzunal (III) as yellowish orange-brown, elongated needles (0.1 g.), m.p. 250~251°.

The precipitate from the remaining acetone solution was extracted with benzene (400 cc.). After filtration of the insoluble residue (A) the filtrate was allowed to stand overnight. The crystalline mass that separated out was collected and recrystallized from benzene to give juzunol (IV) (50 mg.) as yellowish orange-brown needles, m.p. >300°.

The remaining benzene solution was chromatograghed on alumina (10 g.) and developed with

benzene. The product obtained from benzene eluate was recrystallized repeatedly from acetone to give damnacanthal (I) as yellow needles (0.3 g.), m.p. 211~212°.

The above-mentioned insoluble residue (A) was dissolved in acetone and chromatograghed on filter paper (Toyo Roshi No. 50) using BuOH saturated with 27% NH_4OH as the developing solvent. Two spots, Rf 0.38 and 0.55, separated after 2 hr.'s development. From the upper spot, damnacanthol (II) was obtained as yellow needles (0.15 g.), m.p. >300°(decomp.), by recrystallization from acetone, and from the lower spot, juzunol (IV) (0.15 g.), as yellowish orange-brown needles by recrystallization from acetone, m.p. >300°(darkens, decomp.).

It was shown that *Damnacanthus major* Sieb. et Zucc. var. *parvifolius* Koidz. contains the same components described above in almost similar yield.

Juzunal (III, XXXIII)--Soluble in 5% $NaHCO_3$ giving brown orange solution. It gives a brown color with $FeCl_3$ in EtOH, a brown-red color with $Mg(AcO)_2$, and a brown-orange color with NH_4OH-saturated BuOH, which was used as a developing solvent for paper chromatography giving a spot of Rf 0.25. *Anal.* Calcd. for $C_{16}H_{10}O_6$: C, 64.43; H, 3.24. Found : C, 64.64; H, 3.01.

Dimethyl Ether of (III)—Juzunal was methylated by boiling with Me_2SO_4 and K_2CO_3 in acetone for 8 hr. to yield yellow needles, m.p. 227~228°. *Anal.* Calcd. for $C_{18}H_{14}O_6$: C, 66.25; H, 4.32. Found : C, 66.64; H, 4.58.

2,4-Dinitrophenylhydrazone of (III)—Yellowish brown needles (from nitrobenzene), m.p. 300°(decomp.). *Anal.* Calcd. for $C_{22}H_{14}O_9N_4$: N, 11.71. Found : N, 11.11.

Norjuzunal (XIV, XXXII)—Juzunal (0.3 g.) was heated with 80% or 85% H_2SO_4(50 cc.) on a water bath for 1 hr. After the mixture was poured into ice-water (200 cc.), the demethylated product, norjuzunal, was crystallized from hydr. acetone and then purified by sublimation at 200~220°/2 mm. Hg to obtain yellowish orange-brown needles of m.p. 266~267°. *Anal.* Calcd. for $C_{15}H_8O_6$: C, 63.39; H, 2.84. Found : C, 63.65; H. 3.01.

Juzunol (IV, XXXIV)—Dissolves in 10% NaOH with yellow-orange color, in conc. H_2SO_4 with red color. It gives a brown color with $FeCl_3$ in EtOH, a brown color with $Mg(AcO)_2$, and a red color with NH_4OH-saturated BuOH. *Anal.* Calcd. for $C_{16}H_{12}O_6$: C, 64.00; H, 4.03. Found : C, 63.64. H, 3.44. IR ν_{max}^{Nujol} cm^{-1} : 3181(OH), 1658 and 1642(non-chelated CO), 1618(chelated CO), 1570 and 1553(phenyl).

Oxidation of Juzunol—Juzunol (50 mg.) was oxidized with active MnO_2 by the same procedure used for the oxidation of damnacanthol (II) into damnacanthal (I). The product was recrystallized from acetone in yellowish orange-brown needles (30 mg.), m.p. 250~251°, undepressed on admixture with natural juzunal. *Anal.* Calcd. for $C_{16}H_{10}O_6$: C, 64.43; H, 3.38. Found : C, 63.84; H, 3.44.

Damnacanthol 2-Butyl Ether—Damnacanthol (II) (0.2 g.) was refluxed gently with BuOH (3 cc.) for 5 hr. The crystals separated out on cooling were recrystallized from BuOH to yellow needles, m.p. 179~180°(decomp. at 217°). *Anal.* Calcd. for $C_{20}H_{20}O_5$: C, 70.57; H, 5.92. Found : C, 70.44; H, 6.30.

Juzunol 2-Butyl Ether—Prepared by the method described above. Orange-brown needles, m.p. 156~157°(decomp. at 210°). *Anal.* Calcd. for $C_{20}H_{20}O_6$: C, 67.40; H, 5.66. Found : C, 67.92; H, 5.65.

Norjuzunol—Juzunol (0.1 g.) was gently refluxed with AcOH (15 cc.) and 48% HBr (10 cc.) for 1 hr. The reaction mixture was poured into water and the precipitate formed was crystallized from hydrous acetone to orange-brown needles, m.p. >300°. *Anal.* Calcd. for $C_{15}H_{10}O_6$: C, 62.94; H, 3.52. Found : C, 63.87; H, 4.24.

Tetraacetate of Norjuzunol—Yellow needles, m.p. 236~237°. It was proved to be identical with 1,3,5-triacetoxy-2-acetoxymethylanthraquinone (XXXI) by a mixed fusion. *Anal.* Calcd. for $C_{23}H_{18}O_{10}$: C, 60.79; H, 3.99. Found : C, 61.01; H, 4.38.

1,3-Di-O-acetyl-4-O-methylpurpurin (XV)—A mixture of 1,3-di-O-acetylpurpurin, m.p. 208~210° (reported[13~14] m.p. 204°) (1.3 g.), acetone (60 cc.), MeI (6 cc.), and Ag_2O (4 g.) was refluxed for 6 hr. From the reaction mixture Ag_2O was filtered off and the filtrate was evaporated. The residue was crystallized from acetone-MeOH (1:1) to yellow prisms (1 g.), m.p. 202~204°. *Anal.* Calcd. for $C_{19}H_{14}O_7$: C, 64.40; H, 3.98. Found : C, 64.81; H, 4.18.

Rearrangement of (XV) to 3-O-Methylpurpurin (XVII)—i) The compound (XV) (0.9 g.) was dissolved in MeOH (90 cc.) with warming, conc. H_2SO_4 (2.5 cc.) was added, the solution was refluxed on a water bath for 1 hr., and poured into ice water. The precipitate was recrystallized from AcOH to orange-red needles, m.p. 236~237°, which was proved to be identical by a mixed fusion with 3-O-methylpurpurin synthesized according to the method of Perkin[13] and Graebe[14]. *Anal.* Calcd. for $C_{15}H_{16}O_5$: C, 66.67; H, 3.73. Found : C, 67.39; H, 3.85.

ii) To a solution of (XV) (0.6 g.) dissolved in MeOH (40 cc.), 10% MeOH-KOH (40 cc.) was added and the

13) A. G. Perkin : J. Chem. Soc., **1929**, 1415.
14) C. Graebe : Ann., **349**, 229(1906).

mixture was refluxed on a water bath for 2 hr. The reaction mixture acidified with AcOH was concentrated and poured into water. The precipitate was crystallized from EtOH to orang-red needles, m.p. 236~238°, which was identical with the above-mentioned 3-O-methylpurpurin by admixture.

iii) The foregoing hydrolyzed compound was acetylated with Ac_2O and fused AcONa or pyridine and furnished yellow needles, m.p. 170~172°, which was identical with 1,4-di-O-acetylpurpurin 3-methyl ether, synthesized by the method of Perkin.[13]

1,3,4-Trihydroxy-2-methylanthraquinone (XX)—A mixture of 4-bromopurpurin[7] (1 g.), m.p. 214~215°, powdered anhyd. H_3BO_3 (4 g.), and conc. H_2SO_4 (15 cc.) was heated in an oil bath at 150~160° for 2.5 hr. When cool, the reaction mixture was poured on crushed ice, the precipitate formed was collected, washed with water, and dried. It was purified by sublimation at 220~230° at 2 mm.Hg and recrystallized from hydr. acetone to fine red needles (0.3 g.), m.p. 266~267°. It gives a violet coloration with conc. H_2SO_4, 10% NaOH, $Mg(AcO)_2$, and NH_4OH-saturated BuOH, and a dark brown color with $FeCl_3$ in EtOH. *Anal.* Calcd. for $C_{15}H_{10}O_5$: C, 66.67; H, 3.73. Found: C, 66.45; H, 3.78. IR ν_{max}^{Nujol} cm^{-1}: 3310 (OH), 1613 (chelated CO), 1575 (phenyl).

1,3,4-Triacetoxy-2-methylanthraquinone (XXI)—3-Methylpurpurin (XX) (0.5 g.) was refluxed with Ac_2O (20 cc.) and fused AcONa (0.5 g.) for 1 hr. Recrystallization from benzene gave yellow needles (0.5 g.), m.p. 213°. *Anal.* Calcd. for $C_{21}H_{16}O_8$: C, 63.63; H, 4.07. Found: C, 64.40; H, 4.02.

1,3,4-Triacetoxy-2-bromomethylanthraquinone (XXII)—To a solution of the compound (XXI) (0.15 g.) dissolved in CCl_4 (4 cc.), N-bromosuccinimide (0.15 g.) and benzoyl peroxide (20 mg.) were added and refluxed for 15 hr. This was worked up as described for preparation of (IX) giving yellow needles (80 mg.), m.p. 219~220°, after recrystallization from AcOH. *Anal.* Calcd. for $C_{21}H_{15}O_8Br$: C, 53.05; H, 3.16. Found: C, 52.60; H, 3.09.

1,3,4-Triacetoxy-2-acetoxymethylanthraquinone (XXIII)—The foregoing bromo derivative (XXII) (0.2 g.) was refluxed with Ac_2O (5 cc.) and fused AcONa (0.2 g.) for 1 hr. The product was purified by recrystallization from benzene to pale yellow needles (0.1 g.), m.p. 182~183°, which was shown to be different from the tetraacetate of natural juzunol, m.p. 236~237°. *Anal.* Calcd. for $C_{23}H_{18}O_{10}$: C, 60.75; H, 3.99. Found: C, 60.45; H, 3.81.

1,3,4-Trimethoxy-2-methylanthraquinone (XXIV)—A mixture of the compound (XX) (0.5 g.), anhyd. K_2CO_3 (5 g.), Me_2SO_4 (2.5 cc.), and acetone (60 cc.) was refluxed for 24 hr. The product was purified by crystallization from MeOH, giving yellow needles (0.4 g.), m.p. 126~127°. *Anal.* Calcd. for $C_{18}H_{16}O_5$: C, 69.22; H, 5.16. Found: C, 69.06; H, 5.00.

1,3,4-Trimethoxy-2-bromomethylanthraquinone (XXV)—The foregoing product (XXIV) (120 mg.) was brominated with N-bromosuccinimide (100 mg.), benzoyl peroxide (20 mg.), and CCl_4 (40 cc.) by heating for 15 hr. The product, on recrystallization from AcOH, gave yellow needles (80 mg.), m.p. 136~137°. *Anal.* Calcd. for $C_{18}H_{15}O_5Br$: C, 55.24; H, 3.83. Found: C, 54.39; H, 3.79.

1,3,4-Trimethoxy-2-acetoxymethylanthraquinone (XXVI)—The foregoing bromide (XXV) (70 mg.) was acetylated as usual, employing Ac_2O (5 cc.) and fused AcONa (70 mg.), and heating for 1 hr. The product, on recrystallization from EtOH, gave pale yellow needles (50 mg.), m.p. 145~146°. *Anal.* Calcd. for $C_{20}H_{18}O_7$: C, 64.86; H, 4.90. Found: C, 64.83; H, 4.95.

1,3,4-Trimethoxy-2-hydroxymethylanthraquinone (XXVII)—The foregoing product (XXVI) (50 mg.) was hydrolyzed with 10% KOH (1 cc.) in acetone (10 cc.) by refluxing for 3 hr. On recrystallization from petr. ether, the product gave pale yellow needles (20 mg.), m.p. 131~132°. *Anal.* Calcd. for $C_{18}H_{16}O_6$: C, 65.85; H, 4.91. Found: C, 66.14; H, 5.04.

1,3,4-Trimethoxy-2-formylanthraquinone (XXVIII)—The compound (XXVII) (0.2 g.) was oxidized with active MnO_2 (1 g.) and AcOEt (60 cc.) as described for damnacanthol (XI). The product was recrystallized from petr. ether to form elongated yellow needles (0.15 g.), m.p. 136~137°, which was much lower than that of juzunal dimethyl ether, m.p. 227~228°. *Anal.* Calcd. for $C_{18}H_{14}O_6$: C, 66.25; H, 4.32. Found: C, 66.49; H, 5.01.

2,4-Dinitrophenylhydrazone of (XXVIII)—Reddish brown needles (from nitrobenzene containing one drop of BuOH), m.p. 245~246°. *Anal.* Calcd. for $C_{24}H_{18}O_9N_4$: N, 11.06. Found: N, 10.99.

1,3,5-Trihydroxy-2-hydroxymethylanthraquinone (Norjuzunol) (XXX)—1,3,5-Trihydroxyanthraquinone (XXIX), prepared by a method similar to that of Venkataraman, *et al.*,[15] Nonomura and Ogata,[16] and Briggs, Dacre, and Nicholls.[17] m.p. >300°(darkens). Trimethyl ether, m.p. 203°; triacetate, m.p. 227~228°.

To a solution of 1,3,5-trihydroxyanthraquinone (XXIX) (250 mg.) in 5% KOH (2.4 cc.), 3.5% HCHO (1 cc.) was added with stirring. The reaction mixture was kept at a room temperature under stirring for 5 hr. After acidification of the reaction mixture with 1% HCl, the separated mass was col-

15) B. S. Joshi, N. Parkash, K. Venkataraman: J. Sci. Ind. Research, India, **13B**, 246, 825(1954).
16) S. Nonomura, T. Ogata: Kumamoto Pharm. Bull., **3**, 9(1958).
17) L. H. Briggs, J. C. Dacre, G. A. Nicholls: J. Chem. Soc., **1948**, 990.

lected, washed with water, and crystallized from hydrous acetone to orange-brown needles (200 mg.), m.p. >300°(darkens). It gives a brown color with $FeCl_3$ in EtOH, an orange-red color with $Mg(AcO)_2$, and a brown-red color with NH_4OH-saturated BuOH, which was used for the developing solvent for paper chromatography giving a spot at Rf 0.45. *Anal.* Calcd. for $C_{15}H_{10}O_6$: C, 62.94; H, 3.52. Found: C, 63.45; H, 3.94.

1,3,5-Triacetoxy-2-acetoxymethylanthraquinone (XXXI)—The foregoing product (XXX) (60 mg.) was heated with Ac_2O (5 cc.) and a drop of conc. H_2SO_4 on a water bath at 60~80° for 3 hr. On crystallization from hydr. acetone the product formed yellow needles (50 mg.), m.p. 236~237°. *Anal.* Calcd. for $C_{23}H_{18}O_{10}$: C, 60.79; H, 3.99. Found: C, 60.96; H, 4.42.

1,3,5-Trihydroxy-2-formylanthraquinone (XXXII)—The hydroxymethyl group of the compound (XXX) was oxidized under the same conditions as in the case of damnacanthol (II, XI). The product crystallized from hydr. acetone to orange-brown needles (quantitative yield), m.p. 266~267°, which was proved to be identical with norjuzunal derived from natural juzunal by mixed fusion and by comparison of their infrared spectra. It dissolves in 10% NaOH and in conc. H_2SO_4 with a red color. It gives a brown color with $FeCl_3$ in EtOH, a deep orange color with $Mg(AcO)_2$, and an orange red color with NH_4OH-saturated BuOH, which was used as the developing solvent for paper chromatography giving a spot at Rf 0.00. *Anal.* Calcd. for $C_{15}H_8O_6$: C, 63.39; H, 2.84. Found: C, 62.82; H, 3.09.

2,4-Dinitrophenylhydrazone of (XXXII)—Red needles (from nitrobenzene containing a drop of BuOH), m.p. >300°(decomp.). *Anal.* Calcd. for $C_{21}H_{12}O_9N_4$: N, 12.01. Found: N, 11.49.

1,3,5-Trimethoxy-2-formylanthraquinone—Methylation of (XXXII) with Me_2SO_4 (2 cc.) and anhyd. K_2CO_3 (2 g.) in acetone (100 cc.) for 8 hr., gave yellow needles, m.p. 227~228°, on recrystallization from hydr. acetone. Admixture with the dimethyl ether of natural juzunal showed no depression of m.p. *Anal.* Calcd. for $C_{18}H_{14}O_6$: C, 66.25; H, 4.32. Found: C, 66.56; H, 4.67.

2,4-Dihydroxy-3-methyl-6′-nitrobenzophenone-2′-carboxylic Acid (XXXVII)—Friedel-Crafts reaction of 2,6-dihydroxytoluene (5.7 g.) and anhyd. 3-nitrophthalic acid (5 g.) with anhyd. $AlCl_3$ (15 g.) in $CHCl_2CHCl_2$ (150 cc.) proceeded under the same conditions as described for (V). On recrystallization from boiling water and then MeOH or hydr. acetone, the product formed colorless rhombic plates (5 g.), m.p. 252°. *Anal.* Calcd. for $C_{15}H_{11}O_7N$: C, 56.78; H, 3.50. Found: C, 56.32; H, 3.70.

2,4-Dihydroxy-3-methyl-6′-aminobenzophenone-2′-carboxylic Acid (XXXVIII)—The nitro compound (XXXVII) (3.1 g.) dissolved in MeOH (60 cc.) was hydrogenated using 20% Pd-C (0.5 g.) as a catalyst until 670 cc. (3 moles) of H_2 was absorbed. After removing the catalyst, the filtrate was distilled off and the residue was recrystallized from MeOH to colorless rhombic plates, m.p. 230°. *Anal.* Calcd. for $C_{15}H_{13}O_6N$: C, 62.71; H, 4.56. Found: C, 62.28; H, 4.61.

1,3-Dihydroxy-2-methyl-8-aminoanthraquinone (XXXIX)—The foregoing amino compound (XXXVIII) (1.7 g.) dissolved in conc. H_2SO_4 (8.5 cc.) was heated in an oil bath at 150° for 20 min. The reaction mixture was poured on crushed ice. The product that separated out was collected, washed with water, dried, and extracted several times with benzene. After removal of the solvent, the residue was recrystallized from acetone to give deep red fine needles (0.6 g.), m.p. 354°(decomp.). *Anal.* Calcd. for $C_{15}H_{11}O_4N$: C, 66.91; H, 4.12. Found: C, 67.12; H, 4.25.

1,3,8-Trihydroxy-2-methylanthraquinone (XL)—To a solution of the foregoing compound (XXXIX) (0.27 g.) dissolved in conc. H_2SO_4 (2 cc.), powdered $NaNO_2$ (108 mg.) was added in small portions under ice-cooling (0° to 2°) with stirring, and the stirring was continued for 10 min. After being allowed to stand at a room temperature for 10 min., the reaction mixture was heated in an oil bath at 120° for 15 min. and poured on crushed ice. The separated mass was collected, washed with water, and recrystallized from benzene to brown-red needles (0.1 g.), m.p. 312°. IR ν_{max}^{Nujol} cm^{-1}: 3417 (OH), 1662 (non-chelated CO), 1618 (chelated CO), 1586 and 1571 (phenyl bands). *Anal.* Calcd. for $C_{15}H_{10}O_5$: C, 66.67; H, 3.73. Found: C, 66.92; H, 3.94.

1,3,8-Triacetoxy-2-methylanthraquinone—Recrystallized from benzene to pale yellow needles (0.1 g.), m.p. 215~216°. *Anal.* Calcd. for $C_{21}H_{16}O_8$: C, 63.63; H, 4.07. Found: C, 63.83; H, 4.00.

1,3,8-Triacetoxy-2-bromomethylanthraquinone (XLII)—A mixture of the foregoing acetate (100 mg.), N-bromosuccinimide (60 mg.), benzoyl peroxide (5 mg.), and CCl_4 (40 cc.) was treated under the same conditions as described for (IX) to afford a bromo compound (XLII) (50 mg.) as yellow needles, m.p. 222°, after recrystallization from AcOH. *Anal.* Calcd. for $C_{21}H_{15}O_8Br$: C, 53.16; H, 3.16. Found: C, 53.83. H, 3.26.

1,3,8-Triacetoxy-2-acetoxymethylanthraquinone—Obtained by treating the foregoing product (XLII) as for (X). Pale yellow needles, m.p. 198~199°, after recrystallization from benzene and then from EtOH. *Anal.* Calcd. for $C_{23}H_{18}O_{10}$: C, 60.79; H, 3.99. Found: C, 60.46; H, 4.02. IR ν_{max}^{Nujol} cm^{-1}: 1770 (phenolic acetate), 1740~1725 (alcoholic acetate), 1662 (non-chelated CO), 1575 (phenyl).

1,3,8-Trihydroxy-2-hydroxymethylanthraquinone (XLIII)—A solution of the foregoing tetraacetate (0.1 g.) in 10% KOH (10 cc.) and acetone (20 cc.) was refluxed gently on a water bath for 5 hr., cooled, and acidified with 3% HCl. The separated mass after removal of acetone was col-

lected, washed with water, dried, and purified by chromatography through anhyd. $CaHPO_4$ using benzene as the developing solvent. From middle band, yellow-orange needles (40 mg.), m.p. 282° (decomp.), were obtained after recrystallization from hydr. acetone. It gives a brown color with $FeCl_3$ in EtOH, a red color with NH_4OH-saturated BuOH and with $Mg(AcO)_2$. It dissolves in conc. H_2SO_4 and in 10% NaOH to give red-violet solution. *Anal.* Calcd. for $C_{15}H_{10}O_6$: C, 62.94; H, 3.52. Found: C, 63.16; H, 3.65. IR ν_{max}^{Nujol} cm^{-1}: 3364 (OH), 1662 (non-chelated CO), 1612 (chelated CO), 1566 (phenyl).

Hydroxymethylation of 1,3,8-Trihydroxyanthraquinone—To a solution of 1,3,8-trihydroxyanthraquinone (0.1 g.) dissolved in 5% KOH, 3.5% HCHO (0.5 cc.) was added with stirring and stirring was continued for 15 hr. The reaction mixture was acidified with 1% HCl and the separated mass was crystallized from a mixture of Me_2CO:AcOH:H_2O (100:1:10) to give fine yellowish orange needles (yield, 80%), m.p. 282°(decomp.). *Anal.* Calcd. for $C_{15}H_{10}O_6$: C, 62.94; H, 3.52. Found: C, 62.84; H, 3.78. All the properties of this compound agreed well with those of the product (XLIII).

The foregoing hydroxymethylated compound (50 mg.) was acetylated with Ac_2O (5 cc.) and fused NaOAc (1 g.) by heating for 2 hr. It was crystallized from EtOH to pale yellow needles, m.p. 198~199°, which was proved to be identical with the acetate of (XLII) by admixture and comparison of infrared spectra. *Anal.* Calcd. for $C_{23}H_{18}O_{10}$: C, 60.79; H, 3.99. Found: C, 61.24; H, 4.38.

1,3,8-Trihydroxy-2-formylanthraquinone (XLIV)—By the same procedure described for (XII), the foregoing hydroxymethylanthraquinone (XLIII) (50 mg.) was oxidized employing active MnO_2 (0.25 g.) in EtOAc (20 cc.) to give an aldehydic compound (XLIV), which formed pale yellow-orange plates, m.p. 215~216°, on recrystallization from hydrous acetone or AcOEt. It gives a brownish red color with $FeCl_3$ in EtOH, a red-violet color with NH_4OH-saturated BuOH, and a pink color with $Mg(AcO)_2$. It dissolves in 10% NaOH to give a purple-red solution and in conc. H_2SO_4 giving a brown color. *Anal.* Calcd. for $C_{15}H_8O_6$: C, 63.39; H, 2.84. Found: C, 63.75; H, 3.35.

2,4-Dinitrophenylhydrazone of (XLIV)—Prepared with Brady's reagent. Recrystallized from nitrobenzene added with a drop of BuOH to orange needles, m.p. 320°(decomp.). *Anal.* Calcd. for $C_{21}H_{12}O_9N_4$: C, 54.32; H, 2.60. Found: C, 54.02; H, 3.11.

The writer expresses his gratitude to Prof. S. Shibata, University of Tokyo, for his kind advices, to Prof. A. Fujita for his encouragement, to Prof. S. Nonomura for permission to carry out researches on this subject, to Dr. H. Kozuma, Shin-nihon-Chisso Minamata Plant, and Dr. O. Tanaka, University of Tokyo, for measurement of infrared spectra, to Prof. E. Sebe, Dr. S. Matsumoto, Assist.-Prof. S. Takahashi, University of Osaka, and Dr. S. Miyano for their valuable advices, to Dr. M. Shimizu, Miike Dye Plant, for the donation of anthraquinone derivatives, to Mr. T. Katsura for collection of the plants, and also to the members in microanalytical laboratory, Faculty of Pharmaceutical Sciences, and Institute of Applied Microbiology, University of Tokyo, and this Faculty for microanalyses.

Summary

Syntheses of damnacanthal, damnacanthol, norjuzunal and norjuzunol, the coloring matters of Damnacanthus spp. were described. The structures of juzunal and juzunol were synthetically established respectively as 2-formyl- and 2-hydroxymethyl-1-methoxy-3,5-dihydroxyanthraquinone. These pigments are the first example of 1,3,5-trihydroxyanthraquinones occurring in nature.

(Received September 23, 1959)