Summary

12-Methyl-1,2,3,4,6,7,12,12*b*-octahydro-2,6-methanoindolo[2,3-*a*]quinolizine (I), which represents the fundamental skeleton of sarpagine type indole alkaloids, was synthesized.

Thus methyl ester (XI) hydrochloride of N-benzyl-l-methyltryptophan was treated with methyl 3-formylpropionate to form tetrahydro-β-carboline derivative (XV), from which 6,10-imino-5*H*-cycloöct[*b*]indole derivative (XVII) was prepared by Dieckmann cyclization with sodium hydride under a certain working condition. This was condensed with methyl bromoacetate followed by ketone fission to yield ketoester (XX), from which N-benzyl group was reductively removed and then converted to N-methyl derivative (XXIII). The ethylene thioketal (XXV) of the latter was desulfurized as usual and the product was reduced by lithium aluminum hydride to furnish the corresponding alcohol (XXVII), which was tosylated with tosyl chloride in pyridine in the cold. Spontaneous quaternization of the tosylate took place forming the cyclized quaternary base (XXVIII), the chloride salt of which was submitted to pyrolysis reaction to produced the desired base (I).

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159. Junzo Shoji,*1 Shoji Shibata, Ushio Sankawa, Heihachiro Taguchi,*2 and Yoshiko Shibanuma*3 : Metabolic Products of Fungi. XXIV.*4 The Structure of Erythroskyrine, a Nitrogen-containing Coloring Matter of *Penicillium islandicum* Sopp.

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More than dozen pigments have been isolated from several strains of *Penicillium islandicum* Sopp grown on the Czapek-Dox medium.[1] Erythroskyrine which was first isolated and studied by Howard and Raistrick[2] is unique among them as it contains nitrogen in its molecule. The present study has been carried out using laboratory cultivation of *P. islandicum* Sopp S-strain which was isolated from molded rice grains. The S-strain produces no anthraquinone pigments as similar as LSHTM BB 233 strain.[2] A minor pigment accompanied by erythroskyrine was removed by $CaHPO_4$-column chromatography, using hexane-acetone (10:1) as the solvent, and the pure erythroskyrine, orange red crystals, m.p. 130~133°, $[\alpha]_D$ +46.9°, thus obtained gave a molecular formula, $C_{26}H_{33}O_6N$, which was revised from the formula, $C_{24}H_{31}O_6N$, proposed by the earlier workers.[2]

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*4 Part XXIII : This Bulletin, **11**, 1576 (1963).

1) S. Shibata : "Recent Progress in Chemistry of Natural and Synthetic Colouring Matters and Related Fields," pp. 147~166, Ref. cited. Academic Press, New York & London, 1962.
2) B. H. Howard, H. Raistrick : Biochem. J., **56**, 216 (1954).

The purity of erythroskyrine was revealed by thin-layer chromatography to give a single spot whose identy with the main spot given by Professor Raistrick's sample of erythroskyrine was established. Erythroskyrine possesses an alcoholic hydroxyl which is proved by the formation of monoacetate whose infrared spectrum shows $\nu_{C=O}$ at 1740 cm^{-1} and ν_{C-O} at 1250 cm^{-1} (alcoholic acetate). The iodine-brown ferric reaction of erythroskyrine is also given by its monoacetate.

The presence of a polyene system in erythroskyrine which is suggested by a blue-violet color reaction with conc. H_2SO_4 is also revealed by the infrared spectrum absorptions at 1584, 1564, 1550, and 1010 cm^{-1} for -CH=CH-, as well as by the nuclear magnetic resonance signals at τ: 3.7~2.9 (10H) (in $CDCl_3$).

On catalytic hydrogenation in ethanol using platinum oxide as a catalyst, erythroskyrine absorbed 5 moles of hydrogen to afford a faint yellowish liquid, decahydro-erythroskyrine, which could not be purified owing to its instability.

The infrared absorption bands and the nuclear magnetic resonance signals of polyene system in the original erythroskyrine disappeared in the decahydro derivative, which gave strong infrared absorption at 2800~3000 cm^{-1} region and nuclear magnetic resonance signals corresponding to 20H at τ : 8.5 region.

Ozonization of erythroskyrine followed by hydrolysis with 2N H_2SO_4 afforded L(+)-N-methylvaline (Ⅱ) (DNP-derivative, m.p. 194°, $[\alpha]_D$ +441° (in $CHCl_3$)), while by the same treatment decahydroerythroskyrine gave L(+)N-methylvaline and decane dicarboxylic acid (Ⅲ), m.p. 126.5°. The β-triketone ring system in decahydroerythroskyrine is suggested by the enol ferric reaction and the formation of a green copper complex salt as well as by the infrared absorptions at 1710 (sh), 1690, 1635 (sh), and 1615 cm^{-1} (in $CHCl_3$), and the ultraviolet absorptions at 225 mμ (log ε 3.86) and 284 mμ (log ε 4.04) (in EtOH).

The β-triketonic ring system in which the N-methylvaline moiety is involved as a lactam linkage has been established by the close resemblance of the ultraviolet and infrared spectra of decahydro derivative with those of tenuazonic acid (Ⅳ) which was obtained from *Alternaria tenuis* Auct. and structurally established by Stickings.[3]

The isopropyl grouping and N-methyl lactam system in erythroskyrine were revea-l ed by the nuclear magnetic resonance signals at τ: 9.05 (3H) doublet (J=7 c.p.s.), τ 8.90 (3H) doublet (J=7 c.p.s.) for $\underline{CH}_3\!>\!CH-$ system and τ : 7.02 (3H), singlet for -CO-N-$\underline{CH}_3$ system.

Decahydro-erythroskyrine was methylated with methyl iodide and potassium carbonate in acetone, and the product was treated subsequently with lithium aluminum hydride in absolute ether to afford a neutral compound, m.p. 64°, a liquid amine and a crystalline amine, $C_{27}H_{51}O_5N$, m.p. 109°, $[\alpha]_D^{25.3}$ +69° (in $CHCl_3$), whose N-$\underline{CH}_3$ signal in the nuclear magnetic resonance spectrum appeared at τ : 7.72 which was shifted from the corresponding signal of erythroskyrine at τ : 7.02 (in $CDCl_3$).

This indicated the conversion of a R-N(Me)-CO- system in erythroskyrine into a R-N(Me)-CH_2- in the crystalline amine. The introduction of a C-CH_3 group by the methylation of decahydroerythroskyrine was shown by the singlet CH_3 signal at τ : 9.07 in the nuclear magnetic resonance spectrum and the negative ferric reaction of the crystalline amine.

According to the nuclear magnetic resonance spectrum, the neutral product, $C_{18}H_{34}O_4$, m.p. 64°, $[\alpha]_D$ +31.1° (in $CHCl_3$), seemed to retain the main part of the structure

3) C. E. Stickings : Biochem. J., **72**, 332 (1959).

of the original erythroskyrine, though it must lose the triketonic ring system probably by thermal decomposition during the process of methylation, since it showed neither nuclear magnetic resonance signals for tertiary C-Me and N-Me nor infrared absorption of carbonyl.

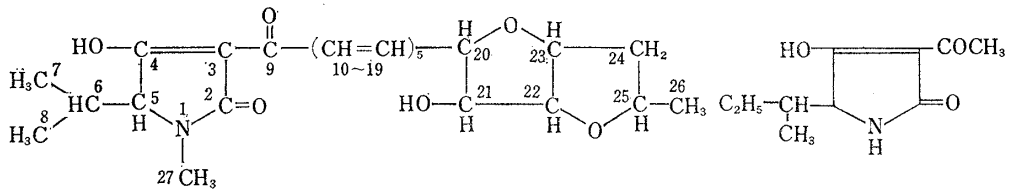
The presence of two hydroxyls, free and hydrogen bonded, respectively, in the neutral product was proved by the infrared spectrum giving doublet bands at 3632 and 3530 cm^{-1} (in CCl_4 using CaF_2 prism).

The neutral compound gave diacetate, $C_{22}H_{38}O_6$, m.p. 29°, which showed no hydroxyl band in the infrared spectrum. On oxidation with the Kiliani reagent, the neutral compound afforded a monohydroxylic acid which was characterised by its monoacetate whose infrared spectrum (in $CHCl_3$) indicated the presence of a carboxyl group (1720 cm^{-1}) and an alcoholic acetate (1740 cm^{-1}). On oxidation with chromic acid at room temperature, the neutral compound gave decane dicarboxylic acid.

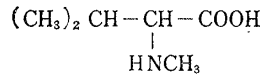
According to these results, it has been suggested that one of the hydroxyls of the neutral compound is a primary and other is in such a position that oxidation is hindered. The analytical results of the neutral compound allocated a bicyclic system involving two ethereal oxygen atoms, one of which is hydrogen-bonded with a hydroxyl. The doublet signal centered on τ : 8.73 of erythroskyrine and the neutral compound indicated the presence of $-O-\underset{|}{CH}-CH_3$ system.

Thus a structure (V) has been proposed for the neutral compound. The infrared absorption (in $CHCl_3$) at 3600 cm^{-1} (free OH) 3535 (bonded OH),[4] as well as the nuclear magnetic resonance spectral data of the model compound, dianhydrosorbitol[5] (VII), m.p. 61~63°, also supported the formula (V).

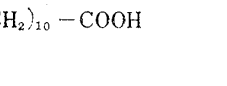
On treatment of diacetate of the neutral compound (VI) with boron trifluoride-ether complex in acetic anhydride[6] followed by deacetylation with lithium aluminum hydride, a tetraol, $C_{18}H_{36}O_5$, m.p. 108°, was afforded. The nuclear magnetic resonance spectrum of the tetraol tetraacetate indicated that one of the ethereal linkages of the original neutral compound was cleaved by boron fluoride and the other ethereal ring was remained unaffected.



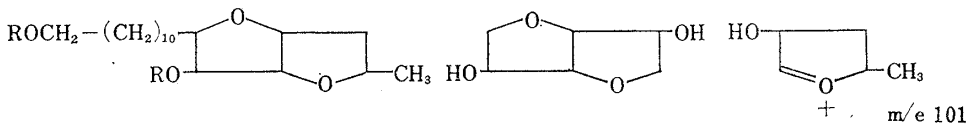
I IV



II



III



V : R=H
VI : R=Ac

VII VIII m/e 101

4) K. W. Buck, A. B. Foster, A. P. Perry, J. M. Webber : J. Chem. Soc., **1963**, 4171.
5) R. Montgomery, L. F. Wiggins : *Ibid.*, **1946**, 390.
6) D. Arigoni, O. Jeger, *et al.* : Helv. Chim. Acta, **44**, 502 (1961).

As one mole of periodate was consumed to oxidise the tetraol, while the neutral componud was not oxidised, the hydroxyl newly formed by the cleavage of ethereal linkage must be located at the adjacent position of the original alcoholic hydroxyl. The mass-spectrum of the neutral compound showed a peak at m/e 101, which would correspond to the ionic fragment (Ⅷ). All the evidences mentioned above showed that the formula (I) represents most reasonably the structure of erythroskyrine.

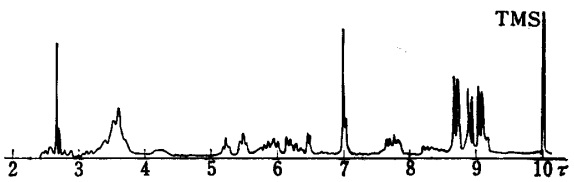


Fig. 1.

The evidence for the ring structure of $C_{(20)}$-$C_{(26)}$ has been provided by the study of nuclear magnetic double resonance spectrum of erythroskyrine measured in deuterochloroform at 100 Mc.p.s. (Fig. 1), and the results of spin-decoupling is shown in Table I.

Table I. Nuclear Magnetic Double Resonance Data of Erythroskyrine

Proton (Position) irradiated (τ)		Proton (Position) observed (τ)		Multiplicity change
25-H	5.75	26-H	8.73	d ⟶ s
		24-H	7.75	(a)*
26-H	8.73	25-H	5.75	(b)*
23-H	5.96	24-H	7.75	(c)*
		20-H	5.25	t ⟶ (−)
22-H	6.21	24-H	7.75	(d)*
		21-H	5.50	t ⟶ d
		20-H	5.25	t ⟶ (−)
21-H	5.50	22-H	6.21	q ⟶ d
6-H	8.37	5-H	6.47	d ⟶ s

*(a) (b) (c) (d) Long range coupling

s: singlet d: doublet t: triplet q: quartet
(−): not decoupled

All the protons of erythroskyrine have been assigned as shown in Table I together with those given in Table Ⅱ.

Table Ⅱ.

τ	Position	τ	Position
9.05 (d)	7-H	7.02 (s)	27-H
8.90 (d)	8-H	3.70~2.9	(10~19)-H
7.05 (s)[a]	21-H		

a) Disappeared when measured on addition of acid.

Experimental

Isolation of Erythroskyrine——The mycelium of *Penicilium islandicum* Sopp Strain-S obtained by the cultivation on Czapek-Dox solution for 3 weeks at 25° in dark was separated from the culture filtrate, rinsed with water and immersed in a mixture of aq. 5% HCl and benzene. The mixture was treated by the method of Raistrick and Howard. The crude extract (25 g.) obtained from the culture

(30 L.) was chromatographed on a column of $CaHPO_4$ using hexane-acetone (10:1) as the developing solvent. The crude pigments separated into four bands, from the bottom to the top: (i) Pale yellow, (ii) orange red, (iii) orange red (main band), and (iv) red. From the main orange red band, crude erythroskyrine was obtained by elution in 10~15% yield of the extract. Repeated crystallization from EtOH afforded, pure erythroskyrine, orange red micro-needles, m.p. 130~133°. *Anal.* Calcd. for $C_{26}H_{33}O_6N$: C, 68.55; H, 7.30; N, 3.08. mol. wt., 455. Found: C, 68.33; H, 7.10; N, 2.99. mol. wt., 455 (Rast). OCH_3: nil. C-CH_3 (Kuhn-Roth) 5.12%. UV λ_{max}^{EtOH} mμ (log ε): 409 (4.45), 260 (3.95). $\lambda_{max}^{0.1N\ NaOH}$ mμ (log ε): 392 (4.78), 260 (4.14). IR max (in $CHCl_3$, 1500~1700 cm^{-1} region): 1550, 1564, 1584, 1612, 1635, 1693.

The pure crystalline erythroskyrine was identified by paper chromatography (Toyo Roshi No. 53. developing with petr. benzine-acetone-H_2O (5:5:3.5 (upper layer)) with the main spot given by Professor Raistrick's sample. The purity of erythroskyrine was established by thin-layer chromatography using Silicagel-G treated with 0.5*N* oxalic acid as adsorbent and a mixture of petr. benzine-acetone-H_2O(5:5:3.5 (uper layer)) as solvent to give Rf 0.29. Erythroskyrine gives an iodine-brown ferric color reaction in ethanolic solution, and dissolves in conc. H_2SO_4 to give a blue-violet color. It is decolorized in Br_2-atmosphere to form a faint yellow liquid.

Erythroskyrine is soluble in acetone, $CHCl_3$, MeOH, EtOH, benzene, AcOH, and pyridine, and less soluble in ether, hexane, and petr. ether.

Erythroskyrine Monoacetate——On acetylation with Ac_2O and AcONa, erythroskyrine afforded monoacetate, which was purified by $CaHPO_4$-chromatography developed with hexane-acetone (10:1), and by recrystallization from hexane-acetone to give orange red micro-needles, m.p. 123°. *Anal.* Calcd. for $C_{28}H_{35}O_7N$: C, 67.6; H, 7.07; N, 2.82. Found: C, 67.42; H, 7.07; N, 2.92. UV λ_{max}^{EtOH} mμ (log ε): 402 (4.65), 260 (4.14), 270 (inflection). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1740 (alcoholic acetate). NMR $\tau_{Si(Me_4)}^{CHCl_3}$: 7.75 (3H, -O-CO-C$\underline{H}_3$). The acetate gives an iodine-brown ferric reaction in ethanolic solution.

Reduction of Erythroskyrine——Erythroskyrine (500 mg.) dissolved in EtOH (50 ml.) was catalytically reduced at 29° using PtO_2 (300 mg.) as a catalyst to absorb 5 moles of H_2 for about 1 hr. On evaporation of the solvent *in vacuo* a faint yellowish liquid was obtained which could not be purified owing its instability.

Copper Salt of Reduced Erythroskyrine——Reduced erythroskyrine (250 mg.) dissolved in EtOH (5 ml.) was titrated with aq. *N* NaOH to pH 7, then treated with a little excess of 0.1*N* copper acetate (14 ml.). The dark green reaction mixture was extracted with $CHCl_3$ to give a green residue on evaporation *in vacuo*, which was dissolved in warm MeOH and diluted with warm water. On cooling, a crystalline green copper salt was separated, which, however, melted at room temperature.

Ozonolysis of Erythroskyrine——Ozonized O_2 was passed through a solution of erythroskyrine (1 g.) in AcOH (30 ml.). After 20 hr., when the orange red color of solution was decolorized, 30% H_2O_2 (7.5 ml.) was added. After standing overnight the excess of H_2O_2 was decomposed by the addition of Pd, and then heated about 50° on a bath. A pale yellow liquid which was obtained on evaporation of solvent was heated on a boiling bath for 8 hr. in 2*N* H_2SO_4 (30 ml.). The brown colored reaction mixture was passed through an IR-4B ion exchange resin column and then through an IR-120 ion exchange resin column, from which amino acids fraction was eluted with 5% NH_3-H_2O.

PPC: Toyo Roshi No. 50: BuOH-AcOH-H_2O (4:1:5) (upper layer).

Rf: 0.53 (Main spot): sprayed with *p*-NO_2-benzoic chloride-pyridine→red, 0.48 (Minor spot): Sprayed with ninhydrin→purple.

Dinitrophenylation of Crude Amino Acids——To a solution of crude amino acid (0.1 g.) in H_2O (5 ml.) and $NaHCO_3$ (0.4 g.), an ethanolic solution of 2,4-dinitrofluorobenzene (0.4 g. in 10 ml.) was added and stirred for 2 hr. in dark at room temperature. The reaction mixture was concentrated and extracted with ether to remove the excess of 2,4-dinitrofluorobenzene, and then acidified with 2*N* HCl. The precipitates thus formed were extracted with $CHCl_3$, and the solution was dried with Na_2SO_4, and then concentrated. From the mixture DNP-amino acids were separated by chromatography on a column of Celite-545 buffered with 0.4*M* phosphate solution at pH 7.0 using $CHCl_3$ as the developing solvent. Dinitrophenol was removed as the first band and from the main band, a crude DNP-amino acid was eluted, which was purified by repeated recrystallization from $CHCl_3$-petr. ether to give orange-yellow leaflets, m.p. 192~193° (decomp.). *Anal.* Calcd. for $C_{12}H_{15}O_6N_3$: C, 48.48; H, 5.09; N, 14.14. Found: C, 48.59; H, 5.11; N, 14.34. $[\alpha]_D^{33.5}$ +440.9° (c=0.615, $CHCl_3$). The DNP-amino acid was proved to be identical with L(+)DNP-N-methylvaline by a mixed fusion, a comparison of IR spectra and thin-layer chromatograms (Rf: 0.324; silica gel G; BuOH-AcOH-$CHCl_3$=1.5:1:97.5).

A minor DNP-amino acid was obtained in orange yellow microneedles, m.p. 128~132°, by a repeated recrystallization from $CHCl_3$-ligroin. This DNP-amino acid was proved to be identical with L-DNP-valine by a mixed fusion and a comparison of IR spectra and thin-layer chromatograms (Rf: 0.56). *Anal.* Calcd. for $C_{11}H_{13}O_6N_3$: C, 46.64; H, 4.63; N, 14.84. Found: C, 46.44; H, 5.01; N, 14.82.

Ozonolysis of Reduced Erythroskyrine——Ozonolysis of reduced erythroskyrine was carried out by the same way described for erythroskyrine. After refluxing with 2*N* H_2SO_4, amorphous precipitates

were filtered off and washed with H_2O. The filtrate was extracted with ether repeatedly to obtain a brown liquid accompanied by colorless crystalline substance which was mixed with the amorphous product and sublimed *in vacuo*. The crystalline sublimate was recrystallized from H_2O to yield micro-needles, m.p. 126°, which were identified as decane dicarboxylic acid by a mixed fusion with the authentic sample. The hydrazide derivate of this acid was colorless micro-needles (from MeOH), m.p. 190°. *Anal.* Calcd. for $C_{12}H_{26}O_2N_4$: C, 55.87; H, 10.14; N, 21.69. Found : C, 55.71; H, 10.19; N, 21.46. The identity of this hydrazide with decane dicarboxylic acid hydrazide was proved by a mixed melting point and IR spectra. L-N-Methylvaline was obtained from the aqueous layer, which was characterized as the DNP derivative which was purified by chromatography on phosphate-buffered Celite 545 column to afford orange yellow leaflets (from $CHCl_3$-petr. ether), m.p. 192~193° (decomp.). The DNP-amino acid was proved to be identical with L-DNP-N-methylvaline by a mixed fusion, and comparison of IR spectra and thin-layer chromatograms.

Methylation of Reduced Erythroskyrine——Reduced erythroskyrine (1 g.) was refluxed with MeI (5 ml), K_2CO_3 (2.5 g.) in acetone (25 ml.) for 7 hr., and the mixture was heated subsequently for 10 hr. after successive addition of MeI (3 ml.) and acetone (5 ml.). The reaction mixture was filtered and washed with acetone, and the filtrate was evaporated *in vacuo* to give a pale yellow liquid which showed no coloration with $FeCl_3$ in EtOH. Purification by high vacuum distillation (1/1000 mm. Hg, 240~250° (bath temp.)) was not successful owing to its instability.

Reduction of Methylated Reduced Erythroskyrine with Lithium Aluminum Hydride——The ethereal solution of methylated reduced erythroskyrine (1 g. in ether 40 ml.) was added with the ethereal suspension of $LiAlH_4$ in 100 ml. ether and refluxed for 30 hr. After decomposing the excess of $LiAlH_4$ by a cautious addition of a few drops of H_2O, 150 ml. of H_2O was added to the ethereal solution. The upper layer was separated and extracted with 2*N* HCl. The ethereal solution was dried on anhydrous Na_2SO_4 and evaporated *in vacuo* to dryness to give a colorless waxy substance which was recrystallized from hexane to afford colorless micro-needles. m.p. 64°. Yield : 170 mg. *Anal.* Calcd. for $C_{18}H_{34}O_4$: C, 68.75; H, 10.90 : mol. wt., 314. Found : C, 68.83; H, 10.91; mol. wt., 314 (Mass-spectrometric). $[\alpha]_D^{26.0}$ + 31.1° (c=0.548, $CHCl_3$). O-CH_3 : nil (micro-Zeisel method, and NMR).

The HCl-layer was alkalised with 2*N* NaOH and extracted with ether to give an oily pale yellow residue on evaporation of the solvent, which was crystallized from acetone-H_2O to afford colorless needles, m.p. 109°. (Yield : 150 mg.). *Anal.* Calcd. for $C_{27}H_{51}O_5N$: C, 69.04; H, 10.95; N, 2.98. mol. wt., 469.7. Found : C, 69.26; H, 10.89; N, 2.99. mol. wt., 462 (Rast). $[\alpha]_D^{25.3}$ +69.0° (c=0.771, $CHCl_3$). O-CH_3 : nil (micro-Zeisel method and NMR) N-CH_3 1 (NMR). It gave an orange red color with Dragendorf's reagent, and a brown violet color with platinum iodide. From the mother liquor of recrystallized amine, a faint yellow oily amine was obtained, which could not be purified by vacuum distillation owing its instability (Yield : about 500 mg.). The same color reactions were given by this liquid amine as observed in crystalline amine. O-CH_3 : nil (micro-Zeisel method).

Acetylation of Neutral Product (Diol)——To a cold solution (6 ml.) of diol (200 mg.) in pyridine Ac_2O (3 ml.) was added under cooling, and the solution was allowed to stand overnight. The reaction mixture was poured in ice water. The precipitates formed were collected and recrystallized from MeOH-H_2O to give colorless needles, m.p. 29°. Yield : 160 mg. *Anal.* Calcd. for $C_{22}H_{38}O_6$: C, 66.30; H, 9.61. Found : C, 66.21; H, 9.52. IR ν_{max}^{Nujol} cm^{-1} : $\nu_{C=O}$ 1748 (-O-$\underline{COCH_3}$), ν_{C-O} 1244; NMR $\tau_{Si(Me)_4}^{CDCl_3}$: 7.93, 7.89 (2 O-$\underline{COCH_3}$).

Acetylation of Diacetyl Diol with Acetic Anhydride and Boron Fluoride——To a cold Ac_2O soln. (3 ml.) of diacetate of the neutral product (diol) (50 mg.) was added 3 drops of BF_3-ether complex under ice cooling, and the mixture was allowed to stand for 1 hr. at room temperature. The resulting faint brown reaction mixture was poured into ice water and after the excess of Ac_2O was decomposed, the reaction mixture was extracted with ether. From the ethereal solution, the solvent was removed *in vacuo*, to give a pale yellow oily residue which was refluxed in dried ether (30 ml.) with $LiAlH_4$ (500 mg.) for 5 hr. After decomposing the excess of $LiAlH_4$ by a cautious addition of a few drops of dil. H_2SO_4, 50 ml. of H_2O was added to the ethereal solution. The ethereal layer was dried over Na_2SO_4 and evaporated to dryness. The colorless precipitates separated in aqueous layer were filtered off, washed with H_2O and dried *in vacuo*. The both products were mixed and recrystallized from $CHCl_3$ to give colorless needles, m.p. 108°. Yield : 30 mg. *Anal.* Calcd. for $C_{18}H_{36}O_5$: C, 65.02; H, 10.92. Found : C, 64.95; H, 10.80.

Oxidation of Neutral Product (Diol) with Chromium Trioxide——A solution of CrO_3 (60 mg.) in AcOH (1.4 ml.) and H_2O (0.1 ml.) was added dropwise to a solution of the neutral product (100 mg.) in AcOH (30 ml.). The mixture was stirred at room temperature for 22 hr. and then poured into ice-water. The precipitates thus formed was extracted with ether and washed with H_2O. The ethereal solution was dried over Na_2SO_4 and evaporated *in vacuo* dryness to give a residue which showed an IR absorption band of COOH at 1720 cm^{-1} (in $CHCl_3$). An excess of CH_2N_2 in ether was added to the ethereal solution of the acid and the mixture was allowed to stand for a few minutes. The residue obtained on evaporation showed an IR absorption band at 1740 cm^{-1} (ester C=O, in $CHCl_3$). The ester was dissolved in EtOH and added with $NH_2OH \cdot H_2O$. The mixture was heated on a boiling water-bath for 1 hr. On concentration

of the reaction mixture, colorless crystals separated out, which on recrystallization from MeOH afforded colorless micro-needles. The product was proved to be identical with decane dicarboxylic acid hydrazide by a mixed fusion and a comparison of IR spectra with the authentic sample.

Dianhydrosorbitol——Dianhydrosorbitol was prepared by refluxing sorbitol in conc. HCl for 24 hr., and the reaction mixture was treated by the method of Montgomery, *et al.* m.p. 60°. *Anal.* Calcd. for $C_6H_{10}O_4$: C, 49.31; H, 6.90. Found : C, 48.93; H, 7.07. IR cm^{-1} ν_{OH} 3600 (free, OH), 3535 (bonded OH). (in $CHCl_3$ dilute solution; CaF_2 prism.).

Estimation of the Consumption of Sodium Periodate during Oxidation of Tetraol and Dion——To a solution of tetraol or diol ($1\sim2\times10^{-5}M$) in MeOH–H_2O (3:2) or AcOH–H_2O, $0.01M$ $NaIO_4$ solution (3 ml.) was added and the mixture was allowed to stand in dark at 25°. The solution was added with saturated $NaHCO_3$ solution (5 ml.), $0.0423M$ standard solution of Na_3AsO_3 and 20% KI solution. After standing for 30 sec., the solution was titrated with I_2 solution ($0.05.M$) using soluble starch solution as the indicator.

IO_4^- Comsumption

Material	Tetraol		
Solvent	MeOH–H_2O	MeOH–H_2O	AcOH–H_2O
Time (hr.)	2	3.5	6
$NaIO_4$ (moles)	1.01	0.7828	0.9064

Material	Diol			
Solvent	MeOH–H_2O	MeOH–H_2O	MeOH–H_2O	AcOH–H_2O
Time (hr.)	6	23	5	4
$NaIO_4$ (moles)	0	0	0	0

Oxidation of Diol with the Kiliani Reagent——To a solution of diol (100 mg.) in acetone (40 ml.), the Kiliani reagent (0.6 ml.) was added dropwise under stirring at room temperature. After 1/2 hr., the reaction mixture was poured in H_2O (50 ml.) and then extracted with ether. Ether was distilled off to give an oily residue which was examined by paper chromatography. (Toyo Roshi No. 50. Solvent : PrOH-conc. NH_4OH (70:30 v/v). Developing agent : Universal indicator). The Rf of oxidative product (0.92) differed from the Rf of decane dicarboxylic acid (0.87). The crude product dissolved in ether was extracted with 5% aq. $NaHCO_3$. Ethereal layer was washed with H_2O, dried over Na_2SO_4 and evaporated. The IR spectrum of the residue was examined to give bands at 3480 cm^{-1} (OH) and 1727 cm^{-1} (C=O) (in $CHCl_3$). The alkaline solution was neutralized with dil. HCl and extracted with ether. The residue obtained by evaporation was acetylated with Ac_2O–pyridine at room temperature. The reaction mixture was poured in H_2O and extracted with ether. The solvent was removed to obtain residue which was examined by IR spectrum in $CHCl_3$. IR $\nu_{max}^{CHCl_3}$ cm^{-1} : 1720 (carboxylic acid), 1740 (alcoholic acetate).

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Summary

The structure of erythroskyrine, $C_{26}H_{33}O_6N$, m.p. 130~133°, $[\alpha]_D$ +46.9°, an orange red pigment of *Penicillium islandicum* Sopp has been elucidated to be represented by the formula (I).

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