55. **Shoji Shibata, Osamu Tanaka, and Isao Kitagawa** : Metabolic Products of Fungi. V.* The Structure of Skyrin. (1).

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As described in the preceding paper,[1] one of the pigments which was isolated from *Endothia parasitica* (Murr.) Anderson et Anderson and *Endothia fluens* Shear et Stevens (syn. *E. radicalis* Fr.), and tentatively named endothianin was established to be identical with skyrin which was obtained from *Penicillium islandicum* Sopp NRRL 1036 and NRRL 1175 by Howard and Raistrick.[2]

The British workers suggested that skyrin having a molecular formula, $C_{30}H_{18}O_{10}$, might have a structure consisting of two molecules of emodin joined together at one of the free position, 1, 3, 6, or 8(methyl in 2) of each moiety,*** because it yielded emodin by the action of alkaline sodium dithionite. Skyric acid, derived from skyrin by the oxidation of methyl group into carboxyl, was also shown to give emodic acid by reductive cleavage.

We also observed that endothianin (skyrin) affords emodin on heating with $AlCl_3$ in benzene, along with a product of unknown structure melting at 210~212° and giving an anthraquinone reaction.[3] In addition to this, emodin was produced by the catalytic reduction of skyrin-pyridine addition product in ethanol-pyridine solution.

As has been shown by Stoll *et al.*[4,5] in the constituents of Senna leaves, sennoside A and B, and their aglycones, sennidins A and B, the meso-linkage of bianthrone is cleaved readily by the action of alkaline sodium dithionite. However, we could not find any adequate example of the reductive cleavage of carbon-carbon linkage of bianthraquinone, though only a brief description was given by Hardacre and Perkin[6] who obtained a small amount of 3-hydroxyanthrone as a by-product of the reduction of 2,2′-dihydroxybianthraquinone with ammonia and zinc.

Therefore, we examined the reductive cleavage of bianthraquinone with sodium dithionite using some model compounds. For such a purpose 2,2′-dihydroxy-, 4,4′-dihydroxy-,[7] and 2,2′-dimethoxy-4,4′-dihydroxy-1,1′-bianthraquinones were prepared. The experiment showed that the compounds having hydroxyl or methoxyl group in the *ortho*-position of the carbon-carbon linkage were broken by sodium dithionite, whereas 4,4′-dihydroxy derivative gave no evidence of cleavage.

2,2′,4,4′-Tetrahydroxybianthraquinone-(1,1′) was synthesized by Scholl[8] by the Friedel-Crafts condensation of 2,2′,4,4′-tetrahydroxybiphenyl with phthalic anhydride and he described a change of color of the bianthraquinone in alkaline sodium dithionite assuming that the stepwise reduction of the quinoid carbonyls might occur.

2,2′-Dimethoxy-4,4′-dihydroxybianthraquinone-(1,1′)(II) which was used for our model experiment was synthesized in a good yield by the Ullmann condensation of 4-bromo-1,3-dimethoxyanthraquinone (I) followed by the partial demethylation with HBr.

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* Part IV. This Bulletin, **3**, 274(1955).

** Hongo, Tokyo (柴田承二, 田中 治, 北川 勳).

*** Bianthraquinone consisting of 2 molecules of emodin joined at the 1-position(methyl in 2) was reported by H. Brockmann, *et al.* (Naturwissenschaften, **38**, 47(1951)).

[1] S. Shibata, T. Murakami, O. Tanaka, G. Chihara, M. Sumimoto: This Bulletin, **3**, 274(1955).

[2] B. H. Howard, H. Raistrick: Biochem. J., **56**, 56(1954).

[3] S. Shibata, O. Tanaka, G. Chihara, M. Mitsuhashi: This Bulletin, **1**, 302(1953).

[4] A. Stoll, B. Becker: Fortschr. Chem. org. Naturstoffe, **7**, 248 (Julius Springer, Wien (1950)).

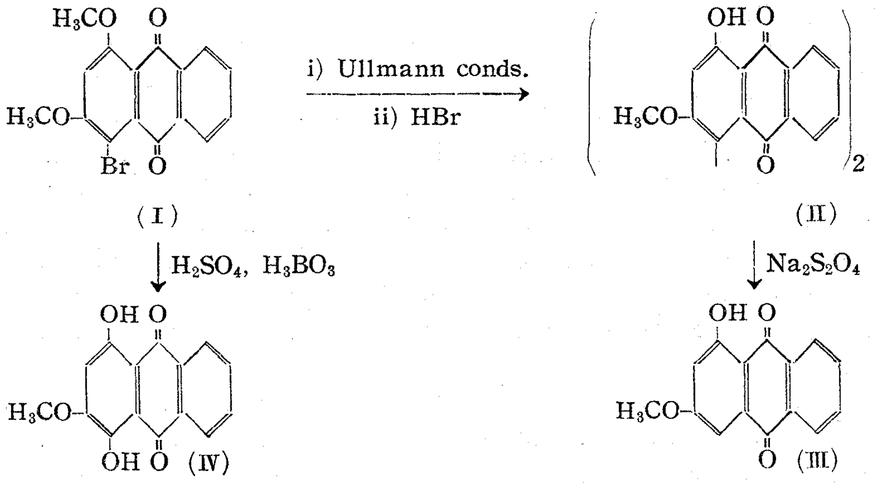
[5] A. Stoll, A. Helfenstein: Helv. Chim. Acta, **33**, 313(1950).

[6] R. W. Hardacre, A. G. Perkin: J. Chem. Soc., **1929**, 180.

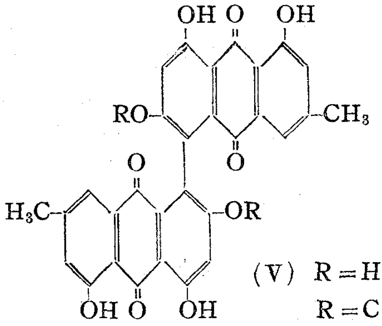
[7] R. Scholl, Ch. Seer: Ber., **44**, 1091(1911).

[8] R. Scholl, W. Neovius: *Ibid.*, **44**, 1075(1911).

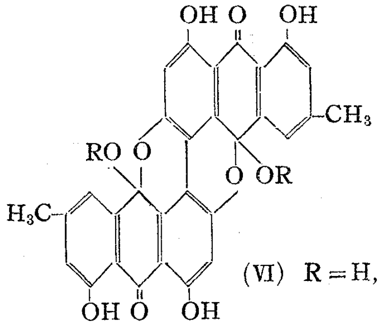
The position of the bromine in the anthraquinone ring was confirmed by deriving it to the 3-methyl ether of purpurin (IV) by the action of conc. H_2SO_4 and boric acid.



Thus the reductive fission of the carbon-carbon linkage of bianthraquinone with alkaline sodium dithonite was proved to occur under a suitable effect of the substitutent, and consequently the bianthraquinone structure of skyrin seems not to be improbable. However, it should be noted that there are many possibilities of linking two emodin moieties to form the skyrin molecule. As mentioned in the preliminary report, γ-coccinic acid methyl ether was obtained by the oxidation of methyl ether of endothianin (=skyrin). This might exclude the possibilities of linkage at the 1- or 3-position of the anthraquinone moiety, and strongly suggests that the linkage would be present between 8 and 8′ positions (methyl in 2) of the emodin moities of skyrin. The structural formula (V, R=H) would, therefore, be most probable for skyrin.



(V) R=H or R=CH_3



(VI) R=H, R=CH_3 or C_2H_5

Howard and Raistrick[2)] described in their report that skyrin forms a methylated or ethylated compound with methanol or ethanol either on long standing in the alcoholic solution or by boiling a solution of hexaacetylskyrin in the alcohol containing conc. H_2SO_4. Dimethyl- and diethyl-skyrins having two alkoxyls are insoluble in aqueous carbonate showing the blocking of β-hydroxyls and they are hydrolyzed readily with alkali to regenerate skyrin. Howard and Raistrick recognized that there is no suitable example of analogous behavior of β-methoxyanthraquinone against alkaline hydrolysis, nevertheless they considered that methylation might occur in the β-hydroxyls of skyrin. It should be noted that methoxyl in the β-position of bianthraquinone, in particular, resists demethylation reaction as shown in 2,2′-dimethoxybianthraquinone-(1,1′), which is not hydrolysed either by the action of HBr or pyridine hydrochloride at 190°.[9)]

It seems necessary, therefore, to clarify this peculiar behavior of skyrin reacting with alcohol. On methylation with diazomethane in ethereal solution, skyrin forms a

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9) H. Brockmann, *et al.*: Chem. Ber., **84**, 865(1951).

dimethyl ether, m.p. above 360°(blackens from 330°), which differs obviously, in the coloration with conc. H_2SO_4, from the methylated derivative of skyrin obtained by Howard and Raistrick.

The methyl ether of skyrin formed by the action of diazomethane should be a genuine β-methyl ether, because it resists alkaline hydrolysis and gives physcion (emodin 7-monomethyl ether) by reductive cleavage with sodium dithionite in alkali.

On the other hand, Howard and Raistrick described that skyrin is dimorphic, crystallizing either as orange rods or hexagonal yellow plates. The former is obtained when crude skyrin is slowly recrystallized from ethereal solution and gives a deep cherry-red coloration with conc. H_2SO_4. Within a few seconds the color changes into a stable emerald green. The latter form which is obtained by evaporating a hot saturated solution in ethanol or acetone exhibits an emerald green coloration in conc. H_2SO_4, immediately.

The above authors considered that the emerald green coloration of skyrin solution results from interaction between β-hydroxyls on two emodin moieties.

We interpret the phenomenon mentioned above from a different point of view basing on the fact of obtaining two different types of methyl ether of skyrin.

According to our observations, two forms of crystals of skyrin which show different behaviors in coloration with conc. H_2SO_4 seem not to be the result of dimorphism of crystals but the isomerisation of their structure. It is noted that the conversion of the form of skyrin scarecely occurs in the highly purified crystals under the conditions indicated by Howard and Raistrick.

To explain the behavior of skyrin with alcohol, we tentatively advance a structural formula (VI ; $R=CH_3$ or C_2H_5) for the isomerized form of skyrin alkyl ethers, though at present the evidences available are not satistfactory.

The infrared spectrum of skyrin does not give a non-chelated carbonyl band in 5.8~6.0μ region[10] but shows a chelated hydroxyl band in 3.18 μ (Fig. 1-A) while emodin obviously shows both non-chelated and chelated carbonyl bands (Fig. 1-D). The configuration of skyrin shown in formula (V; R=H) would result in the chelation between β-hydroxyls and quinoid carbonyls of each emodin moiety. As was expected, the methyl ether of skyrin formed by the action of diazomethane gives the non-chelated carbonyl band (5.97 μ) in its infrared spectrum (Fig. 1-B). As a model compound, 2,2′-dihydroxy-bianthraquinone (1,1′)(VII) was studied by its infrared spectrum in which both chelated (6.03 μ) and non-chelated carbonyl (5.95 μ) bands were

Fig. 1-A

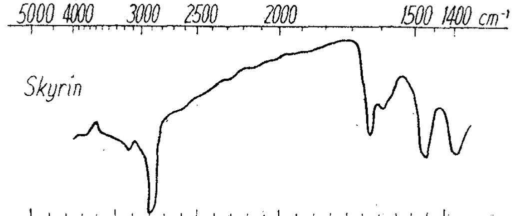


Fig. 1-B

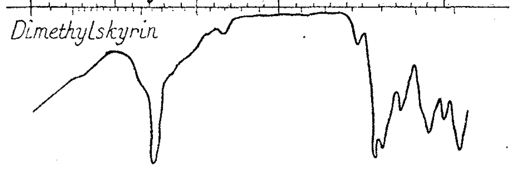


Fig. 1-C

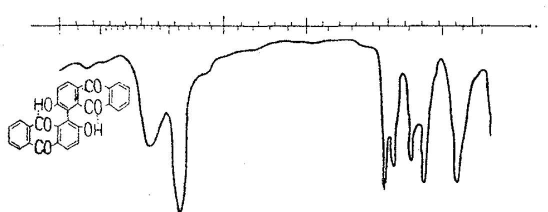
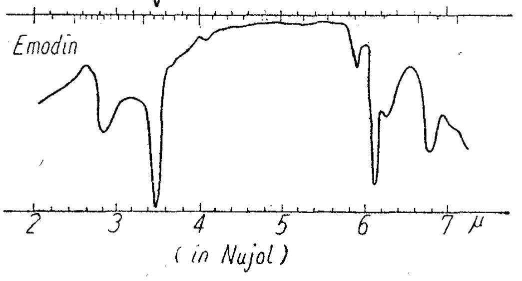


Fig. 1-D



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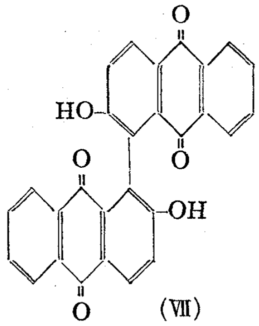
[10] M. St. C. Flett : J. Chem. Soc., **1948**, 1441.


(VII)

observed and the band corresponding to the β-hydroxyl group had shifted to 3.10 μ by the bathochromic effect of chelation (Fig. 1-C).

Although the optical activity may be expected in skyrin by the restricted rotation of its molecule in analogy with that observed in some bianthraquinone and bianthracene derivatives by Bell *et al.*,[11,12] optical rotation has not been observed in skyrin and its derivatives so far as were examined.

We wish to thank Professor H. Raistrick, London School of Hygiene & Tropical Medicine, for his encouragement for this work. The determination of the infrared spectra was made by Mr. S. Tanaka, Dept. Applied Chemistry, and the members of Takamine Laboratory, Sankyo Co., Ltd. The microanalyses were carried out by the members of microanalytical laboratories of the Institute for Infectious Diseases, National Institute of Health, Tokyo, and this Institute to whom our thanks are due.

Experimental

Reductive Cleavage of Skyrin*

Action of $Na_2S_2O_4$ on Skyrin—To a warm solution of skyrin (100 mg.) in *N* Na_2CO_3 (20 cc.), aq. solution of $Na_2S_2O_4$ (3 g. in 20 cc.) was gradually added. The color of the solution changed to light orange red. The reaction mixture was cautiously neutralized under cooling. The orange precipitate formed was taken up in ether and the solvent was distilled off to give orange needles, m.p. 255~257°. Yield: 92 mg. It gives no melting point depression on admixture with emodin. The identity was also proved by the Rf value on the paper chromatogram. Skyrin was subjected to the reductive cleavage with $Na_2S_2O_4$ giving emodin either in 5% NaOH solution or pyridine solution.

Catalytic Reduction of Skyrin—Pyridine addition product of skyrin absorbed hydrogen when it was shaken in pyridine-EtOH (1:1) solution with Pd-black as a catalyst. A syrupy residue obtained by evaporation of the solvent from the filtrate of the reaction mixture was purified by sublimation in high vacuum to give emodin.

Action of $AlCl_3$ on Skyrin—Skyrin (2.4 g.) was suspended in abs. benzene (50 cc.) and added with powdered anhyd. $AlCl_3$ (10 g.). The mixture was stirred under heating at 90~100°, when HCl evolved. After heating for 6 hrs., the benzene solution was decanted to separate a syrupy dark-colored substance, which was shaken with ice-water and then with EtOH-KOH. The alkaline layer was separated and acidified, and extracted with benzene and ether. From the carbonate-soluble portion of the benzene-ether extract, emodin was obtained (yield: 240 mg.), and orange yellow crystals, m.p. 210~212°, was isolated from the carbonate-insoluble portion (yield: 200 mg.). The product, m.p. 210~212°, gives a similar property with physcion but shows a depression of melting point by the admixture with physcion. The chemical structure of the product is still obscure.

Reductive Cleavage of the Model Bianthraquinones

2,2′-Dihydroxybianthraqninone-(1,1′)—Prepared by the procedure given by Perkin, *et al.*[6] It forms yellow amorphous powder melting at about 350°, which is sparingly soluble in almost all oragnic solvents tested except pyridine. It gives a reddish purple solution with conc. H_2SO_4 and dissolves in 2 *N* Na_2CO_3 to give a deep red solution. 2,2′-Dihydroxybiantharquinone-(1,1′) (100 mg.) purified through the diacetate was dissolved in aq. 5% NaOH (20 cc.) and added with 1% aq. $Na_2S_2O_4$ solution (4 cc.). The red colored reaction mixture was acidified with HCl and the precipitate formed was extracted with ether and purified by the usual procedure. The yellow crystals crystallized from glacial HOAc give m.p. 299.5°, undepressed by admixture with 2-hydroxyanthraquinone (m.p. 302°). The Rf values of both compounds on the paper chromatogram developed by acetone-petroleum benzine (b.p. 60°)-water (2 : 2 : 1) are 0.8, whereas 2,2′-dihydroxybianthraquinone-(1,1′) shows Rf 0.25. Diacetate: Bright yellow prisms, m.p. 278~279°. *Anal.* Calcd. for $C_{32}H_{18}O_8$: C, 72.45; H, 3.40. Found: C, 72.32; H, 3.7.

2,2′-Dimethoxybianthraquinone-(1,1′)—Prepared from the dihydroxy compound by reacting with diazomethane in dioxane or Me_2SO_4 and anhyd. K_2CO_3 in acetone. The crude product was chromatographed on an alumina column using $CHCl_3$ as a developing solvent. Crystals from nitro-

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11) F. Bell, D. H. Waring: *Ibid.*, **1949**, 2689.
12) F. Bell, W. H. D. Morgan: *Ibid.*, **1950**, 1963.
* Skyrin used for this work was isolated form *Endothia parasitica* (Murr.) And. et And.

benzene–BuOH mixture form yellow plates, m.p. 343~345°.*

4,4′-Dihydroxybianthraquinone-(1,1′)—Prapared by the procedure given by Scholl.[7] 4,4′-Dihydroxybianthraquinone-(1,1′) was purified through dimethyl ether, to yellow prisms, m.p. 377~381°, crystallized from nitrobenzene–BuOH mixture. The product obtained by reacting alkaline $Na_2S_2O_4$ on 4,4′-dihydroxybianthraquinone-(1,1′) does not melt below 360°, and gives Rf 0 on the paper chromatogram using petroleum benzine as a developing solvent while 1-hydroxyanthraquinone gives Rf 0.9 and m.p. 190°.

2-(2′,4′-Dimethoxy-5-bromobenzoyl)benzoic Acid—The solution of 4-bromoresorcinol dimethyl ether (b.p.$_3$ 100°) (25 g.) and phthalic anhydride (17 g.) in CS_2(100 cc.) was added with anhyd. $AlCl_3$(33 g.) under vigorous stirring when a sticky brownish solid separated out. After heating for 7 hrs. at 70~75°(bath temp.), the reaction mixture was cooled and added with ice and dil. HCl to decompose the $AlCl_3$-addition product. The unreacted 4-bromoresorcinol dimethyl ether was recovered by steam distillation. A dark colored resinous residue was dried and recrystallized from benzene or toluene to colorless prisms, m.p. 181~182°. Yield: 8 g. *Anal.* Calcd. for $C_{16}H_{13}O_5Br$: C, 52.36; H, 3.56. Found: C, 52.31; H, 3.68.

4-Bromo-1-hydroxy-3-methoxyanthraquinone—A mixture of 2-(2′,4′-dimethoxy-5-bromobenzoyl)-benzoic acid (3 g.), 10% fuming $H_2SO_4 \cdot SO_3$(110 cc.), and H_3BO_3(1.2 g.) was heated with stirring at 75° (bath temp.) for 45 mins. The reaction mixture was poured into ice-water and the yellow precipitate formed was extracted with ether. The ethereal solution was shaken with aq. $NaHCO_3$ and subsequently with aq. NaOH. From the bicarbonate-soluble portion about 1 g. of the starting material was recovered and from the caustic alkali-soluble portion orange-yellow crystals, m.p. 235~237°, were obtained by recrystallization from glacial HOAc. It gives an orange coloration with $Mg(OAc)_2$ in EtOH. Rf 0.9 on the paper chromatogram developing with MeOH-saturated petroleum benzine (b.p. 60~80°). *Anal.* Calcd. for $C_{15}H_9O_4Br$: C, 54.05; H, 2.73. Found: C, 54.25; H, 2.61.

Purpurin 3-methyl ether and a remarkable amount of sulfonated product were formed when the reaction temperature was raised above 100° or the time of the reaction was prolonged, or in the case of using $H_2SO_4 \cdot SO_3$ of higher concentration than 10%.

Methylation of 4-bromo-1-hydroxy-3-methoxyanthraquinone with Me_2SO_4 and K_2CO_3 in acetone yielded 4-bromo-1,3-dimethoxyanthraquinone (I), yellow crystals, m.p. 150~152° (from benzene).

2,2′,4,4′-Tetramethoxybianthraquinone-(1,1′)—A mixture of 4-bromo-1,3-dimethoxyanthraquinone (1 g.), activated Cu-bronze,[13] and naphthalene (2 g.) was heated at 225° (bath temp.) for 2.5 hrs. The reaction mixture was extracted with EtOH repeatedly to remove naphthalene. The residue was dried and extracted with $CHCl_3$. The $CHCl_3$ solution was concentrated and chromatographed through activated alumina. MeOH was added to the concentrated eluate and the solution was concentrated to obtain orange yellow needles, m.p. 316~317° (yield: 450 mg.), which were crystallized from BuOH containing a small amount of nitrobenzene.

2,2′-Dimethoxy-4,4′-dihydroxybianthraquinone-(1,1′) (II)—2,2′,4,4′-Tetramethoxybianthraquinone-(1,1′) (150 mg.) was boiled with a mixture of glacial HOAc (10 cc.) and HBr (b.p. 124°) (5 cc.) when it formed a red solution from which orange crystals separated out during the reaction. After boiling for 40 mins. the crystals were collected, washed, dried, and recrystallized from BuOH containing nitrobenzene to form orange needles, m.p. 334~336°. *Anal.* Calcd. for $C_{30}H_{18}O_8$: C, 71,14; H, 3.55. Found: C, 71,13; H, 3.73.

2,2′-Dimethoxy-4,4′-dihydroxybianthraquinone-(1,1′) (30 mg.) dissolved in warm 5% aq. NaOH (5 cc.) was added with aq. solution of $Na_2S_2O_4$(1 g.), when the original red color of the solution changed to orange. Yellow precipitate obtained by acidification was extracted with a mixture of ether and benzene and purified by recrystallization from benzene to yellow crystals, m.p. 192~194°, which was identified with xanthopurpurin 3-methyl ether by a mixed fusion and the Rf value of paper chromatogram.

Formation of Purpurin 3-Methyl Ether (IV) from 4-Bromo-1,3-dimethoxyanthraquinone (I)—A mixture of 4-bromo-1,3-dimethoxyanthraquinone (I) (100 mg.), melted H_3BO_3 (200 mg.), $CuSO_4$ (200 mg.), and conc. H_2SO_4(5 cc.) was heated at 110~130° (bath temp.) for 1.5 hrs. The reaction mixture was poured on ice and the red precipitate formed was extracted with $CHCl_3$ and washed with aq. bicarbonate solution. The solvent was distilled off and the residue was recrystallized from benzene and sublimed to red crystals, m.p. 234~236°, which showed a strong green fluorescence in HOAc solution and were identified with purpurin 3-methyl ether by a mixed fusion and paper chromatography.

Oxidative Reaction of Skyrin

Oxidation of Skyrin Hexamethyl Ether with CrO_3—A solution of CrO_3(6 g.) in aq. HOAc (1 : 5)

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* Brockmann prepared this compound by the Ullmann condensation of 1-iodo-2-methoxyanthraquinone and gave m.p. 361°.

13) E. C. Klerderer: J. Am. Chem. Soc., **55**, 4225 (1933).

(30 cc.) was added to skyrin hexamethyl ether (1.5 g.) suspended in a mixture of HOAc and Ac_2O (20 cc. of each) during 25 mins. After the vigorous reaction ceased, water (100 cc.) was added to the reaction mixture, and extracted repeatedly with ether and $CHCl_3$. The solvent was removed to leave a brownish oily substance which formed needles, m.p. 165~166°, by sublimation at 130° in vacuum and recrystallization. The product which exhibits a positive phthalic acid reaction gives a methyl ester, m.p. 85°. Mixed fusion of the methyl ester with dimethyl ester of γ-coccinic acid methyl ether (m.p. 85°) showed no depression of m.p.

Oxidation of Hexaacetylskyrin with CrO_3 (Formation of Hexaacetylskyric Acid)—To a mixture of hexaacetylskyrin (550 mg.), HOAc (12 cc.), and Ac_2O (12 cc.), a solution of CrO_3 (1.2 g.) dissolved in HOAc was added at 50~60°, during 40 mins. Finally, the temperature was raised to 90°. The reaction mixture was poured into water and yellow precipitate that formed was separated. Recrystallization gave golden yellow needles, m.p. 250° (decomp.), which dissolve in aq. bicarbonate solution. *Anal.* Calcd. for $C_{42}H_{26}O_{20}$: C, 59.29; H, 3.05; 6 CH_3CO–, 30.34. Found: C, 59.02; H, 3.33; CH_3CO–, 30.96.

Dimethyl Hexaacetylskyrate—Prepared by the action of CH_2N_2 from hexaacetylskyric acid as yellow needles (from aq. HOAc), m.p. 255° (decomp.). *Anal.* Calcd. for $C_{44}H_{30}O_{20}$: C, 60.13; H, 3.41; 2 CH_3O, 7.06. Found: C, 59.70; H, 3.73; CH_3O, 7.36, 6.86.

β,β'-Dimethylskyrin (Skyrin β,β'-Dimethyl Ether Prepared with Diazomethane) (V R=CH_3)—Skyrin suspended in abs. ether was added with ethereal solution of CH_2N_2 and the mixture was allowed to stand overnight. Orange yellow crystals collected were extracted repeatedly with $CHCl_3$ to separate insoluble unchanged skyrin.

$CHCl_3$ extract was shaken thoroughly with aq. Na_2CO_3 solution until the carbonate layer became colorless. The $CHCl_3$ extract was evaporated and the residue was recrystallized from nitrobenzene to orange crystals which do not melt below 360° (darken from 325°) and show a similar color reaction given by physcion. It forms a red purple solution with conc. H_2SO_4, whose color does not change to an emerald green on standing. It differs in following respects with dimethylskyrin* obtained by Howard and Raistrick[2] by the action of MeOH and conc. H_2SO_4 from hexaacetylskyrin: On heating with 5% aq. NaOH in a boiling water bath for 1 hr., it is recovered unchanged, and does not undergo any reaction with MeOH and conc. H_2SO_4. It yields physcion (emodin 7-methyl ether), m.p. 202~204°, on reductive cleavage with alkaline $Na_2S_2O_4$. *Anal.* Calcd. for $C_{32}H_{22}O_{10}$: C, 67.84; H, 3.89. Found: C, 67.86; H, 4.07.

It resists demethylation reaction and the result of Zeisel's methoxyl estimation is unsatisfactory.

β,β'-Dimethylskyrin Tetraethoxycarbonyl Ether—β,β'-Dimethylskyrin was reacted with $ClCO_2Et$ and pyridine by the usual method. The product was recrystallized from an acetone-96% EtOH mixture to yellow crystals, m.p. 247~249°. *Anal.* Calcd. for $C_{44}H_{38}O_{18}$: C, 61.83; H, 4.45. Found: C, 61.58, 61.88; H, 4.48, 4.49.

Summary

The structure of skyrin and its reductive cleavage with $Na_2S_2O_4$ giving emodin were discussed using some model compounds, 4,4′-dihydroxy-, 2,2′-dihydroxy-, and 2,2′-dimethoxy-4,4′-dihydroxy-bianthraquinones-(1,1′). The latter two compounds were subjected to the cleavage reaction with $Na_2S_2O_4$. Dimethyl ether of skyrin prepared by the action of methanol and conc. H_2SO_4 was proved to be different from dimethyl ether prepared with diazomethane, the latter yielded physcion by reductive cleavage. It was considered that the latter is a genuine dimethyl ether of skyrin while an isomerized form was suggested for the former compound designating pseudoskyrin dimethyl ether. A formula, 2,4,5,2′,4′,5′-hexahydroxy-7,7′-dimethylbianthraquinone-(1,1′) was proposed for skyrin.

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* We propose to designate this methylated compound as dimethylpseudoskyrin or pseudoskyrin dimethyl ether (see Part VI on p. 284 of this Bulletin).