lieferte ein kristallines Pikrat : Nadeln aus MeOH, Schmp. 174～176°. $C_{25}H_{30}O_{10}N_4$ (Va-Pikrat)—Ber.: C, 54.94; H, 5.53; N, 10.25. Gef. : C, 54.94; H, 5.43; N, 10.35. Bei weiterem Eluieren mit Me_2CO wurden Kristalle vom Schmp. 186～189° erhalten. 1.21 g (Ausbeute 38.1%). Umkristallisation aus AcOEt ergab Kristalle vom Schmp. 188～189°. Sie waren identisch mit Vb.

Epimerisierung von Va——150 mg Va wurden 24 Std. mit 300 mg $Al(iso\text{-}OPr)_3$ und Toluol unter Rückfluß erhitzt. Die übliche Aufarbeitung ergab Kristalle vom Schmp. 187～189°, die identisch mit Vb waren. 114 mg (Ausbeute 76%).

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Zusammenfassung

Es wird die Reduktionsreaktion des C_6-Carbonyls von Morphinalkaloid mittels *T. sanguinea* beschrieben. Die Umsetzung des Dihydrocodeinons ergibt die entsprechenden epimeren 6-Ole. Bei der Umsetzung des Dihydrothebainon-methyläthers bzw. des 4-Desoxydihydrothebainons verläuft aber diese Reduktion nicht glatt. Sinomenin und seine Derivate lassen sich auch durch diese Mikroorganismen nicht angreifen.

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UDC 581.134:547.972

63. Masayasu Kurono, Koji Nakanishi, Keiko Shindo[*1], and Masaru Tada :

Biosyntheses of Monascorubrin and Monascoflavin.

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Birch *et al.* showed in 1958[1] that the mould pigment screlotiorin (structure revised in 1959[2]) is built up from acetic acid and formic acid units according to the acetate theory.[3] Monascorubrin[4,5] and monascoflavin[6,7] are pigments closely related to screlotiorin (azaphilone pigments), and the present studies were undertaken to assist structural studies and to clarify the biosynthetic relation between the two pigments.

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1) A. J. Birch, P. Fitton, E. Pride, A. J. Ryan, H. Smith, W. B. Whalley : J. Chem. Soc., **1958**, 4576.

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Experimental

The activity measurement was carried out with a Nihon Musen Aloka-DC-8 gas-flow counter (back ground, 1.86±0.07 cpm.; disk area, 4.15 cm.^{2}), and the results are expressed in terms of the relative molar activity value, which is proportional to the number of labelled carbons in the molecule. This was calculated by multiplying the counts per 100 sec./cm.^{2} by the number of carbon atoms in the molecule. The samples were combusted by the Van Slyke method[8] and then made into the $BaCO_3$ disks with infinite thickness. No correction was made since the counting was carried out to the value of 10,000 counts.

Selection of Strain——The following fifteen strains[*3] were tested for the production of monascorubrin and monascoflavin with Czapek's medium, Nishikawa's medium[9] and various natural media. Only No. 15 gave reasonable yields of monascorubrin and monascoflavin. No. 1, *Monascus major* Sato, 4485; No. 2, *M. anka* Nakasava et Sato, 4478; No. 3, *M. araneosus* Sato, 4482; No. 4, *M. rubiginosus* Sato, 4484; No. 5, *M. purpureus* Wentii, 4513; No. 6, *M. ruber* Van Tieghen, 4492; No. 7, *M. anka* Nakasawa et Sato, No. 8, *M. anka* Var *rubberus* Sato; No. 9, *M. pilosus* Sato; No. 10, *M. purbigerus* Sato; No. 11, *M. serorubescens* Sato; No. 12, *M. vitreus* Sato; No. 14, *M. serotinus* Sato; No. 15, *M. species* (Yukawa strain).

^{14}C-Monascorubrin (I) and ^{14}C-Monascoflavin (IV)——The Yukawa strain was grown on Nishikawa's medium at 30°, pH 4.5. After 6 days, 0.05 mc. of aqueous $^{14}CH_3COONa$ was added to two flasks each containing 500 ml. of medium. The flasks were equipped with cotton filter tubes that were connected to alkali wash bottles. The cultivation was continued for additional 5 days in the mentioned system; by this time the pH of the medium had a value of 7.1. The dried mycelia (21.5 g.) were percolated with Et_2O, the extract was condensed and the crude crystals were separated from the oil by filtration. One recrystallization from EtOH yielded 800 mg. of monascorubrin contaminated with monascoflavin. This was treated with 3.0 g. of carrier monascorubrin and recrystallized from EtOH, upon which 2.9 g. of labeled monascorubrin was obtained, m.p. 132~133° (decomp.), found r.m.a. 290.8. The mother liquid (20 ml.) was treated with 2.0 g. of carrier monascoflavin and the remaining monascorubrin was selectively destroyed by adding 0.5 ml. of 30% H_2O_2[6] and leaving the solution overnight at room temperature. One recrystallization from EtOH afforded 1.9 g. of labelled monascoflavin, m.p. 142~143° (decomp.), found r.m.a. 195.0.

Kuhn-Roth Oxidation——Kuhn-Roth oxidation of monascorubrin (580 mg.) yielded 2.44 moles of AcOH, which was converted into the Li salt by carrying out the final titration with 0.1*N* LiOH. The salt was pyrolysed at 380~390° *in vacuo*, to yield Me_2CO and Li_2CO_3; the latter was converted into Ba_2CO_3 for counting, found r.m.a. 11.29, i.e., relative ^{14}C-value 0.43. The Me_2CO was converted into CHI_3, which was sublimed at 110°, 18 mm.Hg, before the Van-Slyke combustion; found r.m.a. 15.60, relative ^{14}C-value 0.59.

Similarly, Kuhn-Roth oxidation of monascoflavin (480 mg.) gave 2.44 moles of AcOH, which was converted into Li_2CO_3, found r.m.a. 7.96, relative ^{14}C-value 0.41, and CHI_3, found r.m.a. 10.84, relative ^{14}C-value 0.56.

Monascamine (II)——Monascorubrin (300 mg.) suspended in 5 ml. of EtOH was rapidly dissolved in 10 ml. of conc. NH_4OH with vigorous stirring at 0°. After 5 min., the deep purple solution was diluted with 30 ml. of H_2O and weakly acidified with 2*N* HCl at 0° to give precipitates.

Recrystallization of the well-washed precipitates from EtOH gave monascamine (160 mg.), m.p. 192° (decomp.), found r.m.a. 290.8, relative ^{14}C-value 11.00.

Monascaminone (III)——To a solution of monascamine (300 mg.) in 5 ml. of pyridine containing 2 g. of Zn dust there was added dropwise 9 ml. of AcOH while color changed to orange. After 20 min., the mixture was poured into a large amount of H_2O (100 ml.) with vigorous stirring. The orange solid was dissolved in hot tetrachloroethane, and separated from unchanged Zn dust by filtration; recrystallization from the same solvent gave monascaminone (50 mg.) as white needles, m.p. 186° (decomp.), found r.m.a. 291.5, relative ^{14}C-value 11.03.

Result and Discussion

It has been reported[10] that several strains of the *Monascus* genus produce the two pigments. Although screening tests showed that those preserved in Japan had mutated

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8) M. Calvin : "Isotopic Carbon," p. 92 (1955), John Wiley and Sons, New York.

9) H. Nishikawa : J. Agric. Chem. Soc. Japan, 2, 688 (1926).

10) A.D.G. Powell, A. Robertson, W.B. Whalley : "Chem. Soc. Spec. Publ.," **No. 5**, p. 27 (1956).

and did not produce the required pigments, a strain from Okinawa trivially designated Yukawa was found to produce the pigments; the yield was not high but was sufficient to meet the demands for carrying out the usual degradations. The fungus was cultured on a medium containing $^{14}CH_3COONa$ and the labeled pigments were degraded as shown in Chart. Since the r.m.a. of monascorubrin, which would contain eleven labeled carbon atoms according to theory was 290.8, this was taken as being equivalent to a value of eleven, and the r.m.a. of other degradation products are expressed in terms of this value. In the following this vallue will be named the relative ^{14}C-value for the sake of convenience.

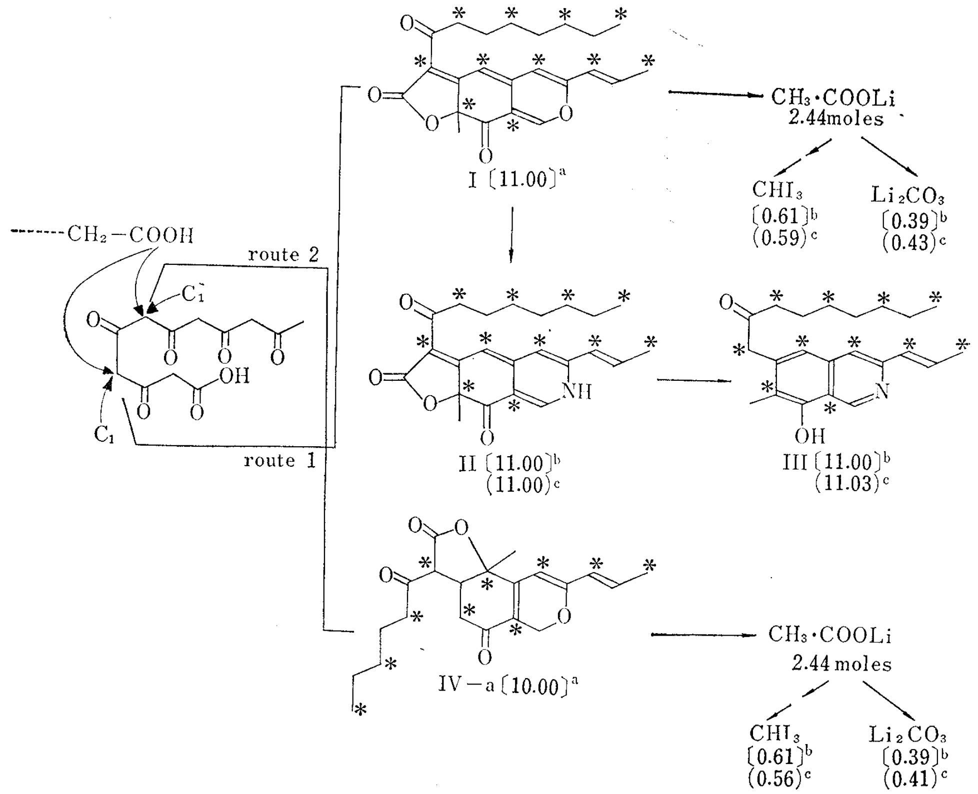

Chart 1. Biogenetic Route of Monascorubrin and Monascoflavin, and Relative ^{14}C-Values

* : carbon originating from methyl group

a : standard b : calculated c : found

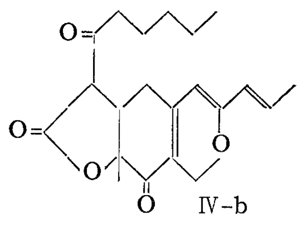
IV-b

Fig. 1.

The distribution pattern of monascorubrin shown in formula I is in good agreement with experimental findings. Kuhn-Roth oxidation gave 2.44 moles of acetic acid*[4] from the three C-methyl groups. The relative ^{14}C-value of the iodoform and lithium carbonate derived from the acetic acid according to standard procedures[11] were in agreement with the distribution pattern of labeled carbon atoms shown in formula I.

However, since the contribution to the Kuhn-Roth C-methyl value differs according to the nature of groups, it was assumed that of the 2.44 moles of acetic acid, 0.85 mole had been derived from the propenyl group,[12] 0.95 mole from

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*[4] The Kuhn-Roth oxidation was carried out for a period slightly longer than usual so as to ensure that acetic acid was the sole oxidation product (checked by paper chromatography).

11) J.W. Cornforth, G.D. Hunter, G. Popjak : Biochem. J., **54**, 597 (1953).

12) R. Kuhn, H. Roth : "Quantitative Organische Microanalyse," Ed. by F. Pregl, p. 246 (1935), Julius Springer, Berlin.

the angular methyl[12] and the remaining 0.64 mole from the *n*-heptyl side-chain. Thus the relative ^{14}C-value for iodoform and lithium carbonate are $(0.85+0.64)/2.44=0.61$ and $0.95/2.44=0.39$, respectively. These compare well with the experimental values of 0.59 and 0.43. The conversion of monascorubrin (I) into monascamine (II) merely involves the replacement of -O- by -NH-, and that of monascamine (II) into monascaminone (III) is a decarboxylation involving the non-labeled lactone ring.[4] No change in the relative ^{14}C-value should be expected from these conversions, and this is again supported by experimental findings.

The r.m.a. of monascoflavin (IV-a or IV-b) was 195.0, and similar to the case of monascorubrin, this was assigned a relative ^{14}C-value of ten according to the distribution pattern which contained ten labelled carbon atoms. The oxidation similarly yielded 2.44 moles of acetic acid, and the relative ^{14}C-value was calculated as described above. Since in the present case the angular methyl group is attached to a vinylogous α-ketolactone, instead of the ketolactone in monascorubrin, it has been assumed that the former unit would also afford 0.95 mole of acetic acid.

It is conceivable that acetic acid enters the TCA-cycle with subsequent randamisation of activity between its C-1 and C-2 atoms. Thus, although randamisation of labelling is known to occur in moieties derived from sugar, it is well-established that the extent is rather low and that it does not affect the experimental results in the case of moieties derived from the linear condensation of acetic acid units, the polyketomethylene chain or its variant.

The structures of monascorubrin (I),[4,5] and monascoflavin (IV-a),[6] or (IV-b)[6,7] are thus consistent with biosynthetic results, and it is indicated that the two pigments are synthesized through closely related mechanisms. The ring systems come from a common polyketomethylene precursor, and the distinction between the two pigments arises from the difference in the direction of attachment and length of the side-chain polyketomethylene (cf. Chart). Route 1 leads to monascorubrin and route 2 leads to monascoflavin, and it appears that the direction of attachment is governed by the point of attachment of the extra angular methyl group derived from methionine or its equivalent. In the case of the alternate structure (IV-b) for monascoflavin, it would be formed via route 1 in a manner parallel to that of monascorubrin.

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Summary

The biosyntheses of monascorubrin and monascoflavin by a strain of *Monascus* genus have been studied and the two pigments have shown to be derived from the linear condensation of acetic acid units. The results confirm the proposed structure of both pigments.

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