[Chem. Pharm. Bull. 13(8)1015~1017(1965)]

UDC 581.13 : 547.565.2

Biosynthesis of Lichen Depsides, Lecanoric Acid and Atranorin

Lichens are known to produce several phenolic compounds, such as depsides, depsidones, and anthraquinones, which show a systematic structural interrelation suggesting that they are occurring by a mutual biogenetic scheme such as acetate-malonate pathway.*[1]

However, biosynthetical experiment of lichen substances has been regarded to be of great difficulty mainly due to the extremely slow growth of lichens and unsuccessful large scale laboratory cultivation of intact lichens or their mould components.

Our experiment has been designed at first to test the turnover rate of carbon metabolism in lichen. ^{14}C-Labeled carbon dioxide was filled in a dessicator in which a small thallus of *Parmelia tinctorum* was placed on a Petri dish and allowed to stand for one week.

The radioactivity incorporated in the lichen thallus during this period was unexpectedly high. Accordingly, we carried out the biosynthetical experiment by spraying aqueous solutions of acetate-1 ^{14}C and formate-^{14}C (0.1 mc. each) over the fresh lichen thallus.

After one week, the lichen thallus was dried and then extracted with ether. The ethereal extract was recrystallized from a mixture of ethanol-benzene to afford lecanoric acid, m.p. 175°, and a small amount of atranorin, m.p. 196°. Chloroatranorin, m.p. 208° was isolated from the mother liquor of crystallization, by silicic acid chromatography, using chloroform as the solvent.

The radioactivity of lecanoric acid was measured with Tricarb 314 EX type liquid scintillation counter, using the solution in ethanol (1 ml.) mixed with toluene (14 ml.) containing PPO (0.4%) and POPOP (0.01%). Atranorin and chloroatranorin were dissolved in a mixture of benzene (1 ml.) and toluene scintillator (14 ml.) to measure the radioactivity.

TABLE I.

Precursor	Specific activity d.p.m./m*M*	
Compounds	Acetate-1 ^{14}C	Formate-^{14}C
Lecanoric acid	2.9×10^5	0
Atranorin	8.8×10^5	1.7×10^4
Chloroatranorin	3.6×10^5	4.5×10^3

The results given in Table I show that acetate is a mutual precursor of these lichen depsides, whereas formate is incorporated only into atranorin and chloroatranorin to reveal that it participates as a C_1-fragment to form CH_3 and CHO groupings. The higher radioactivity of atranorin in comparison with that of lecanoric acid seemed to suggest the C_1-fragment incorporation occurs prior to the completion of aromatic ring, but in accord with the larger content of lecanoric acid in the lichen, it would also be probable that it is resulted by the dilution of radioactive lecanoric acid by the non-labeled lecanoric acid which has been formed prior to the ^{14}C-administration.

Degradation of ^{14}C-labeled lecanoric acid was carried out as follows to show the location of radioactivities in the molecule.

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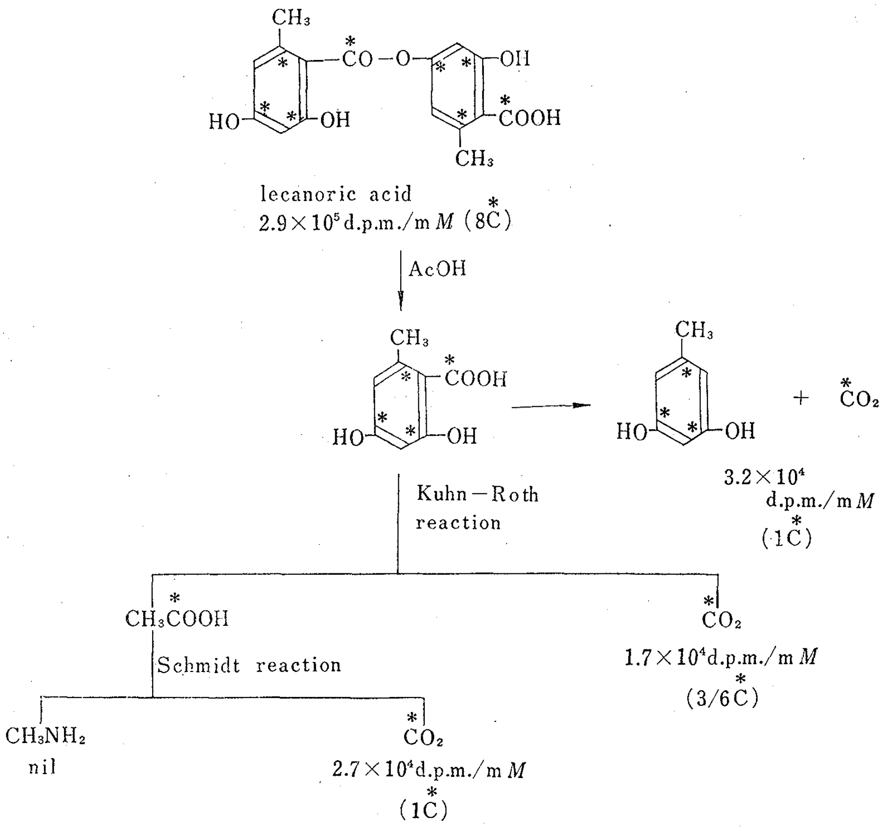
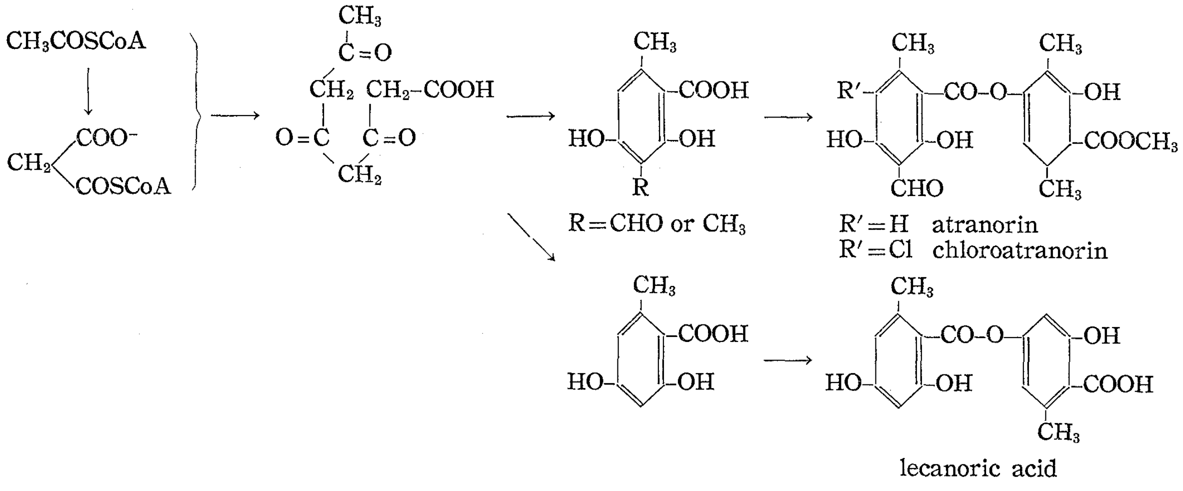
*[1] S. Shibata has proposed to classify the lichen substances on the biogenetical basis. "Beitrage zur Biochemie und Physiologie von Naturstoffen," Professor K. Mothes 65th Birthday Anniversary volume (in press, 1965).



The participation of 1 mole of acetate and 4 moles of malonate in orsellinic acid in Penicillium Sp. has been established by Mosbach,[1a)] and by Bentley and Keil.[1b)] The present experiment has revealed that this scheme can be extended to the depsides in lichens. The co-occurrence of orcinol and β-orcinol type depsides in the same lichen as has happened in this case would be noted from biogenetical view point.



When our experiments were under progress, Mosbach[2)] has reported the biosynthesis of gyrophoric acid, an orcinol type tridepside of *Umbiricaria pustulata*, which has been performed by shaking the fragmented lichen thallus in Czapek-Dox solution

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1) *a*) K. Mosbach: Acta Chem. Scand., **14**, 457 (1960); Naturwiss., **15**, 525 (1961). *b*) R. Bentley, J. G. Keil: Proc. Chem. Soc., **1961**, 111; J. Biol. Chem., **237**, 867 (1962).

2) K. Mosbach: *Ibid.*, **18**, 329 (1964).

containing ^{14}C-labeled ethyl malonate. In his experiment using the lichen thallus packed with the solution of diethyl malonate-^{14}C in a glass cylinder, incorporation of radioactivity into the depside was not observed.

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Received April 22, 1965

[Chem. Pharm. Bull.
13(8)1017~1019(1965)]

UDC 547.857.7.07

The Synthesis of 1-Alkoxy-, 1-Alkoxy-9-alkyl-, and 9-Alkyladenines

Much interest in the recently discovered preferential alkylation of adenine (Ⅳ) on the 3-position[1~4] and of its N-substituted derivatives at the same[*1,1,5,7] or other position[1,4,6~9] stimulated us to investigate the site of alkylation of adenine 1-oxide (I)[10,11] in connection with our current research program. Now we report the synthesis of 9-alkyladenines (Ⅶa, b, c)[12] from Ⅳ, as shown in Chart 1, through the use of the easily removable alkoxy group at the 1-position to orient to the 9-position on alkylation.

Treatment of I with methyl, ethyl iodide, and benzyl bromide in N,N-dimethylacetamide at room temperature furnished the corresponding 1-alkoxyadenine salt (Ⅱ) as the major product (60~80%): 1-methoxyadenine hydriodide (Ⅱa: X=I), m.p. 222° (decomp.); 1-ethoxyadenine hydriodide (Ⅱb: X=I), m.p. 208° (decomp.); 1-benzyloxyadenine hydrobromide (Ⅱc: X=Br), m.p. 214~219° (decomp.). The salts were readily converted in good yields to the corresponding free bases (Ⅲ) by the use of Amberlite IR-45 or basification to pH 7.5 (for Ⅲc): Ⅲa, m.p. 255~257° (decomp.); Ⅲb, m.p. 219° (decomp.); Ⅲc, m.p. 164~166° (decomp.). The 1-alkoxy structure was assignable by the successful catalytic hydrogenolysis of the free bases (Ⅲa, b, c) to adenine (Ⅳ) (84~90%

[*1] In the case of benzylation on N′-benzoyladenine with benzyl bromide, instead of the chloride (Ref. 7), in dimethylacetamide at 90° for 8 hr., we also obtained 3-benzyl-N′-benzoyladenine hydrobromide of m.p. 257~259° (decomp.) in a yield of 57%, which gave (78%) 3-benzyladenine on mild alkaline hydrolysis. A similar observation has been made by B. Shimizu and M. Miyaki (Tetrahedron Letters, No. 25, 2059 (1965).

1) N. J. Leonard, T. Fujii: J. Am. Chem. Soc., **85**, 3719 (1963). (See earlier references cited therein.).
2) C. J. Abshire, L. Berlinguet: Can. J. Chem., **42**, 1599 (1964).
3) N. J. Leonard, R. A. Laursen: Biochemistry, **4**, 354 (1965).
4) M. Asai, M. Miyaki, B. Shimizu: Agr. Biol. Chem., **29**, 170 (1965).
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7) *Idem*: J. Heterocyclic Chem., **1**, 115 (1964).
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12) T. C. Myers, L. Zeleznick: J. Org. Chem., **28**, 2087 (1963), and references therein.