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Hiroshi Mitsuhashi*¹ and Michiko Nomura*² : Studies on the Constituents of Umbelliferae Plants. XII.*³ Biogenesis of 3-Butylphthalide. (1).(Faculty of Pharmaceutical Sciences,*¹ and Radioisotope Center, Hokkaido University*²)

Toki (*Ligusticum actilobum* SIEB. et ZUCC.), Senkyu (*Cnidium officinale* MAKINO) and some other plants belonging to the Umbelliferae contain 3-butylphthalides.^{1~6)} The relative and absolute stereochemistry of ligustilide (I), cnidilide and neocnidilide has been elucidated in these laboratories.^{7~9)} The biogenetic origin of butylphthalides is

Chart 1.

TABLE I.

Compound	Specific activity d.p.m./mmol. BaCO ₃	Relative activity
Sedanonic acid (III)	3.01×10^4	1.00
(V) (C-12)	4.18×10^3	0.14
(VI) (C-11)	3.80×10^4	1.26
(VII) (C-1)	5.26×10^4	1.75

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of considerable interest. One of the possible biogenetic pathways of ligustilide (I) is regarded as that arising from head to tail linkage of acetate units as shown Chart 1.

Feeding experiments using acetic acid (carboxyl- ^{14}C) were carried out on *Levisticum officinale* KOCH. *Levisticum officinale* has been shown suitable for obtaining pure ligustilide compared to other Umbelliferae plants.¹⁰⁾ Radioactive sedanonic acid (III) was prepared by hydrolysis of dihydroligustilide (II) and was degraded as shown in Chart 1. The counting results are shown in Table I. In view of the results described in the Experimental it is suggested that the C-C units of acetate are incorporated into ligustilide without rearrangement.

Experimental

Cultivation of Plants—*Levisticum officinale* KOCH was cultivated in the field for 2 years and the plants used before flowering. Six plants were administered a total of 4 mc. of CH_3COONa (carboxyl- ^{14}C) by the cotton thread method to upper part of main root. The feeding experiments lasted 7 days. Plant materials administered with labeled acetate were minced into small pieces and dried under an IR lamp, weight, 850 g. The dried plant was percolated with petr. ether (b.p. $\sim 50^\circ$). The extract (16 g.) so obtained was dissolved in ether, treated with 2.5% and 5% NaOH solution, washed with H_2O and dried over Na_2SO_4 . Upon evaporation of the solvent a neutral oil (11 g.) was obtained, which was distilled under reduced pressure and the fractions boiling between $70\sim 130^\circ$ at 0.5 mm. Hg collected: Yield 6.03 g. The distillate was chromatographed over a column of silicic acid (Mallincrodt, 100 mesh, for chromatography) (120 g.) with CHCl_3 as the eluting solvent. Ligustilide (I) 1.08 g. was obtained.

Preparation of Dihydroligustilide (II)—Ligustilide (I) (1.08 g.) in ethanol was shaken in hydrogen at 15° and atm. press. with 5% palladized barium carbonate (200 mg.) until 78.8 ml. hydrogen was absorbed. The catalyst was filtered off and the solvent removed *in vacuo*. The residual oil was chromatographed on a silicic acid column (80 g.) with CHCl_3 as an eluting solvent. The fractions with strong UV absorption at $276\text{ m}\mu$ in the eluted CHCl_3 solution, were collected. After removal of the solvent *in vacuo*, dihydroligustilide (II) (592 mg.) was obtained.

Hydrolysis of Dihydroligustilide (II)—Dihydroligustilide (II) (592 mg.) was refluxed 1 hr. with 5% ethanolic KOH (10 ml.), H_2O was added and the solution concentrated *in vacuo*. The resulting aqueous alkaline solution was washed with ether, acidified and extracted with ether. The acid fraction (361 mg.) crystallized from benzene-hexane. Sedanonic acids (III) of m.p. $108\sim 110^\circ$ were obtained.

Measurement of Radioactivity—Radioactive material was burned by the wet combustion method and the resulting BaCO_3 was prepared as a thin film on a copper plate. Radioactivity was measured by the 2π -flowcounter FC-1 (Nihon Musen Co.). The radio chemical purity of sedanonic acid (III) was determined by recrystallization three times. The specific activity was constant after each recrystallizations.

Degradation of Sedanonic Acid (III)—i) Kuhn-Roth oxidation.¹¹⁾ Sedanonic acid (III) 214 mg., 5N CrO_3 (40 ml.), and conc. H_2SO_4 (15 ml.) were placed in the oxidation flask, and the mixture is refluxed over a microflame for 3 hr. The excess CrO_3 was destroyed with hydrazine (0.9 ml.), neutralized with 50% KOH and conc. H_3PO_4 (20 ml.) then added. The flask was set up for steam distillation. The distillate was then titrated with 0.1N NaOH solution to determine the amount of generated AcOH. The crude AcONa was recrystallized from MeOH and ether. Yield 44.6 mg.

ii) Schmidt reaction¹²⁾ of AcONa. A mixture of 41 mg. of AcONa with 50 mg. of NaN_3 and 0.3 ml. of conc. H_2SO_4 was heated for 1 hr. at 75° . The CO_2 evolved was washed with 5% KMnO_4 , and absorbed in $\text{Ba}(\text{OH})_2$ solution. The resulting BaCO_3 was collected. Yield 82.4%. 5 ml. of 5N NaOH solution was added to the decarboxylated solution and the generated CH_3NH_2 was trapped with 0.5N HCl solution. Yield 17.8 mg. (48.5%).

iii) Schmidt reaction of sedanonic acid (III). Sedanonic acid (III) was decarboxylated by application of Schmidt reaction as described above. Yield 29.1%.

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10) H. Mitsuhashi, T. Muramatsu, U. Nagai, T. Nakano, K. Ueno: This Bulletin, **11**, 1317 (1963).

11) R. Kuhn, *et al.*: Angew. Chem., **44**, 847 (1931).

12) E. F. Phares: Arch. Biochem. Biophys., **33**, 173 (1951).