98. Kōtaro Takahashi and Shūichi Miyashita : Usnic Acid. I.
Methyldihydrousnic Acid.

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The structural formula of usnic acid¹⁾ which was previously advanced by Robertson, Schoepf and Asahina has been established by Barton and his co-workers²⁾ by their synthesis of racemi usnic acid which was accomplished by the oxidative condensation of methylphloracetophenone. However, a remarkable difference of the behaviors in the degradation reactions observed between usnic acid and dihydrousnic acid still leaves some room for criticism. Recently, on methylation of usnic acid with dimethyl sulfate and sodium hydroxide, Robertson³⁾ obtained a compound $C_{19}H_{18}O_7$, m.p. 136° . They assumed it to be an enol methyl ether of usnic acid. In this paper, we report on a new product, methylation product of dihydrousnic acid, named methyldihydrousnic acid, and its decomposition reactions. On methylation with dimethyl sulfate and sodium hydroxide, *l*-dihydrousnic acid yielded *d*-methyldihydrousnic acid, $C_{19}H_{20}O_7$ (I), white crystals, m.p. $165\sim 166^\circ$, $[\alpha]_D +218.1^\circ$, which had not a methoxy group by the Zeisel method. This compound was also obtained by catalytic hydrogenation of Robertson's methylation product of usnic acid which had about 0.6 mole of methoxy group by the Zeisel method. During these reactions, the inversion of optical rotation was observed. *d*-Usnic acid ($[\alpha]_D +493.7^\circ$) was methylated to *d*-Robertson's compound ($[\alpha]_D +364^\circ$) which was hydrogenated to *d*-methyldihydrousnic acid ($[\alpha]_D +215.0$), but *d*-usnic acid was hydrogenated to *l*-dihydrousnic acid ($[\alpha]_D -81.4^\circ$) which was methylated to *d*-methyldihydrousnic acid ($[\alpha]_D +218.1^\circ$). From *dl*-dihydrousnic acid, by this methylation, *dl*-methyldihydrousnic acid, m.p. $140\sim 141^\circ$, was obtained.

Methyldihydrousnic acid is soluble in sodium hydrogen carbonate solution and gives red brown coloration with ferric chloride but does not give the coloration with *p*-dimethylaminobenzaldehyde (Ehrlich reaction). By C-methyl determination (Kuhn-Roth), 4 moles of acetic acid were obtained, alike dihydrousnic acid.

The ultraviolet and infrared spectra are shown in Fig. 1 and Fig. 2 with those of

Fig. 1.

- I : Methyldihydrousnic acid
(1.89 mg. in 200 cc. EtOH)
II : Dihydrousnic acid
(3.87 mg. in 520 cc. EtOH)

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1) Y. Asahina, S. Shibata : "The Chemistry of Lichen Substances," Japan Society for the Promotion of Science, Tokyo, Japan, 1954.

2) D.H.R. Barton, A.M. Delforin, O.E. Edwards : J. Chem. Soc., 1956, 530.

3) F.M. Dean, P. Halewood, S. Mongkolsuk, A. Robertson, W.B. Whalley : *Ibid.*, 1953, 1250.

Fig. 2.

(1) Dihydrousnic acid (KBr Pellet)

(2) Methyl dihydrousnic acid (KBr Pellet)

dihydrousnic acid. The remarkable differences between infrared absorption curves of (I) and that of dihydrousnic acid, especially in the region of $1600\sim 1700\text{ cm}^{-1}$ indicate the difference of carbonyl system in these two substances.

(I) yielded bisphenylhydrazone bisanhydride which appeared in two crystal forms, $\text{C}_{31}\text{H}_{28}\text{O}_3\text{N}_4\cdot 2\text{C}_6\text{H}_6$, m.p. 136° and $\text{C}_{31}\text{H}_{28}\text{O}_3\text{N}_4\cdot 2\text{C}_7\text{H}_8$, m.p. $114\sim 116^\circ$, depending on the solvent used for crystallization. The ultraviolet curves of two forms in alcoholic solution were identical.

On treatment with hydroxylamine hydrochloride and sodium acetate in alcoholic solution, (I) gave bisoxime bisanhydride $\text{C}_{19}\text{H}_{18}\text{O}_5\text{N}_2$ (II), m.p. $197\sim 198^\circ$. By oxidation of (II) with hydrogen peroxide, a product $\text{C}_9\text{H}_{11}\text{O}_5\text{N}$ (III), m.p. 216° , was obtained. By the molecular weight determination by the Rast method and acidimetry, (III) was a dicarboxylic acid and gave dimethyl ester by diazomethane, $\text{C}_{11}\text{H}_{15}\text{O}_5\text{N}$, b.p.₁₆ $150\sim 160^\circ$ (bath temp.). By decarboxylation at $220\sim 230^\circ$ and subsequent distillation at $16\sim 17\text{ mm Hg.}$, (III) was converted to a monocarboxylic acid $\text{C}_8\text{H}_{11}\text{O}_3\text{N}$ (IV), m.p. $151\sim 152^\circ$, which was proved to be identical with 3-methyl-5-isopropyl-4-isoxazolecarboxylic acid, synthesized by hydrolysis of oximated ethyl 2-isobutyrylacetoacetate, by the mixed melting point determination, infrared and ultraviolet spectra. Two constitutions are possible to this isoxazole monocarboxylic acid, one of which is 3-methyl-5-isopropyl-4-isoxazolecarboxylic acid (IV) and the other is 3-isopropyl-5-methyl-4-isoxazolecarboxylic acid, but the former is more likely from the view-point of the steric hindrance in the course of the synthesis and (III) has another carboxylic acid at the side-chain of (IV) and may be formulated as (III).

From the fact that methyl groups and acetyl groups in dihydrousnic acid molecule are situated at meta position and a *gem*-dimethyl group is not present, it is reasonable to assume that the methylation of dihydrousnic acid occurred at C-methyl position, resulting in a *gem*-dimethyl group in A-ring of dihydrousnic acid molecule.*² From these results and analytical point of view, the methyl dihydrousnic acid can be formulated as (I).

On oxidation with potassium permanganate in 10% potassium hydroxide solution,

*² part II : This Bulletin, 10, (1962).

4) A. Spassow : Ber., 70, 2381 (1937).

followed by distillation, as in the case of dihydrousnic acid,⁵⁾ (I) yielded 3-methyl-2,6-dihydroxy-acetophenone and a compound, m.p. 235°, faint yellow crystals.

Although some arguments have been presented for the mechanism of the formation of resorcinol type of compounds on Robertson's formula of dihydrousnic acid, an unequivocal experimental proof has not been obtained as yet. But the result that one of resorcinol type of compounds is obtained from methyl dihydrousnic acid indicates that 3-methyl-2,6-dihydroxyacetophenone is derived from one of nucleus of dihydrousnic acid which is not methylated by this method, namely from the B-ring of dihydrousnic acid. (I) did not give the coumaranone derivative⁶⁾ which was obtained from dihydrousnic acid, by the vacuum distillation with calcium chloride or without it.

On acetylation, (I) was converted to a compound (V), $C_{27}H_{20}O_7$, m.p. 172~173°, by obtaining one acetyl group and liberating one mole of water. When treated with concentrated sulfuric acid in the cold or 10% sodium hydroxide on a water bath, liberating one mole of acetyl group, (V) yielded a compound $C_{19}H_{18}O_6$ (VI), m.p. 245~246°, which corresponds to anhydromethyl dihydrousnic acid which was reacylated to (V).

Chart 1.

The authors are now studying on this anhydromethyl dihydrousnic acid and will report shortly on its constitution and decomposition reactions.

Experimental*3

Methylation of Dihydrousnic Acid—To a solution of 15 g. of *l*-dihydrousnic acid in 600 cc. of 2N NaOH, 45 cc. of dimethyl sulfate was dropped for 30~40 min. under vigorous stirring at 15~20°. After 1 hr stirring, the solution was neutralized with 10% HCl. The precipitate was filtered off and recrystallized from EtOH giving white crystals, *d*-methyl dihydrousnic acid (I), m.p. 165~166°. Yield, 11 g. It was soluble in $NaHCO_3$ solution and gave red brown coloration with $FeCl_3$ in EtOH solution. It had no methoxy group and gave no melting point depression on admixture with hydrogenated product of Robertson's methylation product of usnic acid, and both had the same IR absorption

*3 Ultraviolet absorption spectra were taken in ethanol solution using Hitachi EPU-2A spectrophotometer. Infra-red absorption spectra were taken using Nippon Bunko Model IRS Infra code.

5) Y. Asahina, M. Yanagita: *Yakugaku Zasshi*, **59**, 699(1939).

6) *Idem*: *Ibid.*, **59**, 702 (1939).

curves. UV $\lambda_{\max}$ $m\mu$ (ϵ): 230 (16,000), 274 (18,700). $[\alpha]_D^{25}$: +218.1° ($c=1.16$, CHCl_3). Anal. Calcd. for $\text{C}_{19}\text{H}_{20}\text{O}_7$: C, 63.33; H, 5.59. Found: C, 63.32, 63.06; H, 5.42, 5.51.

dl-Methylidihydrousnic Acid—m.p. 140~141°. This was obtained by the methylation of *dl*-dihydrousnic acid. Anal. Found: C, 63.28; H, 5.74.

Catalytic Hydrogenation of Robertson's Methylation Product of Usnic Acid—*d*-Rotatory substance (4 g.), dissolved in tetrahydrofuran (30 cc.), was hydrogenated with Pd-black. After half an hour's shaking, when 370 cc. (ca. 1.4 moles) of H_2 was absorbed, the solvent was removed *in vacuo* and the residue was recrystallized from MeOH to give white crystals, m.p. 166°. $[\alpha]_D^{25}$: +215.0° ($c=1.275$, CHCl_3). Anal. Calcd. for $\text{C}_{19}\text{H}_{20}\text{O}_7$: C, 63.33; H, 5.59. Found: C, 62.98; H, 5.52.

C-Methyl Determination of (I) and Dihydrousnic Acid by the Kuhn-Roth Method—To neutralize AcOH obtained from 0.0988 g. of (I), 9.587 cc. of 0.1N KOH was required. Found: C-CH₃, 3.88 mole. To neutralize AcOH obtained from 0.1013 g. of dihydrousnic acid, 10.30 cc. of 0.1N KOH was required. Found: C-CH₃, 3.52 moles.

Bisphenylhydrazone Bisanhydride of (I)—A mixture of 3 g. of (I), 3 g. of phenylhydrazine hydrochloride, 1.7 g. of anhyd. AcONa and 60 cc. of EtOH was boiled on a water bath for 3 hr. After evaporation of EtOH *in vacuo*, water was added and the precipitate was recrystallized. From benzene it forms white crystals, m.p. 135~136° (decomp.). Anal. Calcd. for $\text{C}_{31}\text{H}_{28}\text{O}_3\text{N}_4 \cdot 2\text{C}_6\text{H}_6$: C, 78.15; H, 6.10; N, 8.48. Found: C, 78.01; H, 6.18; N, 8.66 (dried on CaCl_2 at ordinary pressure). UV $\lambda_{\max}$ $m\mu$ (ϵ): 209 (28,200), 251 (24,700). From toluene it forms white crystals, m.p. 114~116° (decomp.). UV $\lambda_{\max}$ $m\mu$ (ϵ): 210 (35,200), 251 (32,800). Anal. Calcd. for $\text{C}_{31}\text{H}_{28}\text{O}_3\text{N}_4 \cdot 2\text{C}_7\text{H}_8$: C, 78.46; H, 6.44; N, 8.13. Found: C, 78.22; H, 6.44; N, 8.27. On heating with alcoholic HCl on water bath, both were recovered unchanged.

Bisoxime Bisanhydride of (I)—A mixture of 2 g. of (I), 1.0 g. of anhyd. AcONa and 0.9 g. of hydroxylamine hydrochloride in EtOH was refluxed for 4 hr. The solution was concentrated to one-half of its volume and was poured into water. When the precipitate separated out, which was chromatographed on alumina with benzene. The benzene solution was distilled to give crystals (II), m.p. 197~198°. Yield 0.9 g. UV $\lambda_{\max}$ $m\mu$ (ϵ): 212 (21,800), 260 (4,900), 271 (7,100), 330 (5,000). Anal. Calcd. for $\text{C}_{19}\text{H}_{18}\text{O}_5\text{N}_2$: C, 64.40; H, 5.12; N, 7.91. Found: C, 64.36; H, 5.35; N, 8.29.

Oxidation of (II) with Hydrogen Peroxide—To a solution of 3 g. of (II) in 80 cc. of 3% KOH, 30 cc. of 3% H_2O_2 was added. On warming on a water bath at 70~80°, the solution became green after 10~15 min. Then 10 cc. of 3% H_2O_2 was added one time at interval of an hour. After 7 hr. the solution became faint brown. After acidification with dil. HCl and salting out with NaCl, it was extracted with Et_2O . After drying with anhyd. Na_2SO_4 , Et_2O was distilled off to give white crystals (III), m.p. 216° (from water). It was soluble in NaHCO_3 solution with bubbling. UV: $\lambda_{\max}$ 222 $m\mu$ (ϵ 5,800). Anal. Calcd. for $\text{C}_9\text{H}_{11}\text{O}_5\text{N}$: C, 50.70; H, 5.20; N, 6.57. Found: C, 50.33; H, 5.33; N, 6.56. Acidimetry. Calcd. as dicarboxylic acid, COOH %, 42.30%. Found: 42.80%.

Decarboxylation of (III)—On heating 2 g. of (III) at 220~230° (bath temp.) for 10 min., it melted with vigorous evolution, and then was distilled *in vacuo* (bath temp. 160~190°, 16 mm. Hg.) giving oil which solidified in crystals. It was recrystallized from dil. EtOH to give white crystals (IV), m.p. 151~152°, which was soluble in NaHCO_3 solution with evolution. UV: $\lambda_{\max}$ 216 $m\mu$ (ϵ 7,200). Anal. Calcd. for $\text{C}_8\text{H}_{11}\text{O}_3\text{N}$: C, 56.79; H, 6.55; N, 8.28. Found: C, 57.15; H, 6.88; N, 8.53. Molecular weight. Calcd. 169. Found: 175 (Rast. Campher). Acidimetry. Calcd. as monocarboxylic acid, COOH %, 26.62%. Found: 26.60%.

Synthesis of 3-Methyl-5-isopropyl-4-isoxazolecarboxylic Acid—A mixture of 0.4 g. of ethyl isobutyryl acetoacetate and 0.14 g. of hydroxylamine hydrochloride in 3 cc. of EtOH was kept at room temperature overnight. After evaporation of EtOH, a small volume of water was added to give oily substance which was extracted with Et_2O . The Et_2O solution was evaporated and water was added to it. After acidification with dil. HCl, white crystals separated out, which was recrystallized from dil. EtOH, m.p. 151°. It was proved to be identical with (IV) by mixed fusion, IR and UV spectra. UV $\lambda_{\max}$ 216 $m\mu$ (ϵ 6,900). Anal. Calcd. for $\text{C}_8\text{H}_{11}\text{O}_3\text{N}$: C, 56.79; H, 6.55; N, 8.28. Found: C, 56.92; H, 6.51; N, 8.01.

Oxidation of (I). Formation of 3'-methyl-2',6'-dihydroxyacetophenone—A solution of 3.2 g. of (I) in 40 cc. of 10% KOH was diluted to 1 L. with water. To this solution, 100 cc. of 4% KMnO_4 was dropped during 3 hr. under vigorous stirring at room temperature. The filtered alkaline solution was acidified with dil. HCl and then extracted with CHCl_3 . After evaporation of CHCl_3 *in vacuo*, the resulting oil was distilled at 1 mm. Hg. (bath temp. 150~250°). The distillate solidified partially after standing and then was separated into Et_2O -soluble (A) and Et_2O -insoluble (B) fractions.

The Et_2O -soluble fraction (A) was recrystallized from benzene to give yellow needles, m.p. 136~137°, which was identical with 3'-methyl-2',6'-dihydroxyacetophenone by mixed melting point determination, IR and UV spectra. UV $\lambda_{\max}$ $m\mu$ (ϵ): 223 (12,800), 271 (13,900), 353 (3,800). Anal. Calcd. for $\text{C}_9\text{H}_{10}\text{O}_3$: C, 65.05; H, 6.07. Found: C, 65.06; H, 6.09.

The Et_2O -insoluble fraction (B) was recrystallized from a mixture of benzene and petr. ether to

give faint yellow crystals, m.p. 235°. It was soluble in NaHCO_3 solution and gave brown coloration with FeCl_3 . *Anal.* Found: C, 66.76, 66.37; H, 5.44, 5.37.

Acetylation of (I)—A mixture of 10 g. of (I), 100 cc. of Ac_2O and 20 drops of conc. H_2SO_4 was warmed on a water bath for 3 hr. and after cooling, the mixture was poured into ice water. Brown precipitates that separated out were treated with MeOH and then recrystallized from a mixture of EtOH and benzene to yellow crystals, m.p. 172~173°(V). Yield, 4 g. It was optically inactive and gave deep-blue coloration with FeCl_3 . This acetylation did not take place when warmed at 50~60°, as in the case of the acetylation of usnic acid. UV λ_{max} 212.5, 266, 318 and 390 m μ . Molecular weight, calcd. for $\text{C}_{21}\text{H}_{20}\text{O}_7$: 384. Found: 372.7 (Rast. Camphor). *Anal.* Calcd. C, 65.61; H, 5.24. Found: C, 65.76, 65.74, 65.70; H, 5.69, 5.35, 5.12.

Action of Conc. Sulfuric Acid on (V)—To a solution of 50 cc. of conc. H_2SO_4 10 g. of (V) was gradually added at 0~5° and the mixture was allowed to stand for 2 hr. at room temperature and then poured into ice water. Precipitates were recrystallized from benzene to give faint greenish yellow crystals (VI), m.p. 245~246°. Yield, 5.0 g. It was soluble in NaHCO_3 solution and gave blue-green coloration with FeCl_3 in EtOH solution. UV λ_{max} 212, 257, 271.5 and 377 m μ . *Anal.* Calcd. for $\text{C}_{19}\text{H}_{18}\text{O}_6$: C, 66.66; H, 5.30. Found: C, 66.63, 66.67; H, 5.19, 5.33.

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Summary

Methyldihydrousnic acid was obtained by methylation of dihydrousnic acid and its constitution was elucidated as (I). The result that 3'-methyl-2',6'-dihydroxyacetophenone was obtained from methyldihydrousnic acid indicates that the resorcinol type of compound is derived from B-ring of (I). This result also presents the experimental proof of formation of 3'-methyl-2',6'-dihydroxyacetophenone, one of resorcinol type of compound, from B-ring of dihydrousnic acid.

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99. Kōtarō Takahashi, Akie Arai, Kazuko Ōshima, Yoshie Ueda, and Shūichi Miyashita: Usnic Acid. II*¹. Methylusnic Acid.

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Robertson *et al.*¹⁾ obtained methylated usnic acid $\text{C}_{19}\text{H}_{18}\text{O}_7$ (I), m.p. 136° and they assumed it to be an enol methyl ether compound of usnic acid. The facts, obtained during the course of the investigation of the constitution and properties of methyldihydrousnic acid, indicate that the constitution of Robertson's substance can be formulated as (I). So it seemed to be necessary for us to reexamine the constitution and decomposition reactions of Robertson's compound.

On methylation with dimethyl sulfate and sodium hydroxide, *d*-usnic acid afforded a compound (I), m.p. 136°, yellow crystal which was assumed to be identical with the Robertson's compound. (I) gave red brown coloration with ferric chloride and 0.6 mole

*¹ Part I: This Bulletin, 10, 603(1962).

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1) F.M. Dean, P. Halewood, S. Mongkolsuk, A. Robertson, W.B. Whalley: J. Chem. Soc., 1953, 1250.