färbt sich zuerst rötlich und dann tief grün. Es dauerte ca. 1 Stunde. Die Reaktionslösung wurde noch 1 weitere Stunde erhitzt, im Vakuum fast zum Trocknen eingedampft und der Rückstand mit etwa 3-fachem Volumen Wassers zersetzt. Der hierbei ausgeschiedene Niederschlag wurde abgesaugt, in verd. Natronlauge gelöst und mit verd. Essigsäure umgefällt. Nach zweimaliger Wiederholung dieser Operation wurde er aus Methanol-Wasser umkristallisiert. Nadeln vom Zers. Pkt. 237~238°. Die Ausbeute 5.4 g.

Methylderivat : Nadeln, Schmp. 53~55°. Pikrat : Schmp. 194~196°. Eine Mischprobe des Pikrates mit dem Pikrat des *N*-Methoxy-4-chinolonbetain zeigte keine Depression.

Die Durchführung der Versuche wurde durch die Unterstützung des Unterrichtsministeriums für die Förderung der Naturwissenschaften ermöglicht, wofür wir uns zu Dank verpflichtet fühlen.

Zusammenfassung.

4-Oxychinolin-*N*-oxyd gibt bei der Nitrierung mit konz. Salpetersäure in Essigsäureanhydrid-Lösung das 3-Nitroderivat. Beim Erhitzen von 4-Nitrochinolin-*N*-oxyd mit Essigsäureanhydrid wurden eine kleine Menge von 3-Nitro-4-oxychinolin, *N*-Acetoxy-3-nitro-4-chinolonbetain und 4-Oxychinolin-*N*-oxyd erhalten. Die Entstehung von 3-Nitroderivaten wurde durch Hinzufügen von Dimethylanilin unterdrückt, wobei nur das 4-Oxychinolin-*N*-oxyd mit guter Ausbeute entsteht.

(Eingegangen am 28. Februar, 1952)

224. Kyosuke Tsuda* and Masaaki Kawamura** : The Constituents of the Ovaries of Globefish. VII¹⁾. Purification of Tetrodotoxin by Chromatography. (2)²⁾.

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Tawara obtained, in 1909, a crude poison from aqueous extract of globefish ovaries, by precipitation with lead acetate in the presence of ammonia, and named this component tetrodotoxin³⁾. Recently, Yokoo⁴⁾ obtained this poisonous component in a crystalline form (*Anal.* Found : C, 40.4; H, 5.5; N, 11.1; potency, 0.01 γ /g.) by chromatographic purification on alumina column after removing impurities by successive treatment with phosphotungstic acid, mercury picrate, picrolonic acid and phenylhydrazine.

The present authors, as described in Reports I⁵⁾ and IV⁶⁾, isolated mesoinositol, scil-litol and glucose, as impurities, from the crude poison of Tawara's, and also described the purification of this crude poison through partition chromatography on potato starch column, and adsorption chromatography on active charcoal. By these two kinds of chromatography, amino acids and glucose in the crude poison can be removed almost completely.

In the present series of experiments, the crude poison was passed consecutively through these two kinds of chromatographic columns, and the poison thereby obtained was further purified by the use of chromatopile method and recrystallization, from which crystalline tetrodotoxin was obtained.

By consecutive passage through partition chromatographic columns of potato starch

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1, 2) Part VI : This Journal, **72**, 187 (1952). 3) Y. Tawara : This Journal, **29**, 587 (1909).

4) A. Yokoo : J. Chem. Soc. Japan, **71**, 590 (1950).

5) K. Tsuda, M. Kawamura : This Journal, **70**, 70 (1950) ; C. A., **45**, 3950 (1951).

6) K. Tsuda, M. Kawamura : This Journal, **71**, 282 (1951) ; C. A., **45**, 8151 (1951).

and active charcoal, a poison having a potency of 0.2 γ /g. body wt. mouse is obtained. By development of this poison through chromatopile with phenol-water, the poison is concentrated at a point, Rf 0.0~0.1, as in the case of paper chromatography described in the previous report. This crystalline poison, when dissolved in diluted acetic acid, yields uniform crystals upon the addition of alcohol and ether. Recrystallization in the same manner finally yields prismatic crystals (Fig. 1), which are easily soluble in diluted acetic acid, but are sparingly soluble in water, in dehydrated alcohol and in ether. The crystals gradually blacken around 220°, but do not melt even at 300°. The analytical values found are: C, 41.88; H, 5.98; N, 11.81. Its solution in diluted acetic acid of pH 5.0 is fairly stable for a long period of time. The potency is 0.01 to 0.012 γ /g. body wt. of mouse. The yield during the course of this purification is shown in Fig. 2.

Fig. 1. Crystalline Tetrodotoxin

Fig. 2. Purification of Tetrodotoxin.

(Numerals in parentheses show potency of each fraction as minimum lethal dose γ /g. body wt. of mouse).

* This Journal, **70**, 71 (1950).

** This Journal, **72**, 187 (1952).

The various data of the crystals obtained by the present workers are very similar to those of the crystals obtained by Yokoo, but further studies must be made before any fascimilarity or chemical properties can be established.

The present series of experiments were carried out with the Scientific Research Fund from the Ministry of Education for which the authors express their gratitude.

Experimental

Purification of Tetrodotoxin by Chromatopile Method—Circular filter papers of 5.5 cm. in diameter (Toyo Roshi No. 2) are used. Five hundred of these filter papers are stacked, of which, 20 papers between 50th and 70th are allowed to absorb aqueous solution of 500 mg. of tetrodotoxin possessing a potency of 0.2 γ , air dried, and stacked again. This pile is then developed by the ascending system with 90% phenol-water as the partitioning solvent. The papers at Rf 0.0~0.1 are taken out, phenol removed by washing with ether, and digested with cold water. The aqueous extract is evaporated under reduced pressure, washed again with ether, and dried by a reduced pressure.

Potency, MLD 0.12 γ . Yield 200 mg.

Recrystallization—The crystalline powder obtained through the chromatopile is washed several times with absolute alcohol, and the insoluble solid is dissolved in 0.5 cc. of water containing 1 drop of glacial acetic acid. This is filtered, 5 cc. of alcohol added to the filtrate, and the precipitate that separates out is removed by filtration. To this filtrate, 5 cc. of ether is added, allowed to stand overnight, and the globular crystals that separate out are collected and washed with water. These globules, when broken, reveal silky needle crystals. These crystals are dissolved in 0.5 cc. of diluted acetic acid, 5 cc. each of alcohol and ether added, the small amount of precipitate removed by filtration, and the filtrate allowed to stand overnight. The prismatic crystals that separate out are collected, washed with water, and dried in a vacuum desiccator over phosphorus pentoxide. The crystals gradually turn black from around 220°, but do not melt even at 300°. *Anal.* Found: C, 41.88; H, 5.98; N, 11.81.

Summary

Tetrodotoxin, which had previously been purified through chromatographic column of starch and active charcoal, was further purified through chromatopile and recrystallization by which crystalline pure tetrodotoxin, possessing a potency of MLD 0.012 γ /g. body wt. of mouse, was obtained as prismatic crystals.

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225. Masao Tomita and Tadashi Abe: Studies on the Alkaloids of Berberidaceous Plants. IV¹⁾. Alkaloids of *Mahonia Fortunei* Fedde.

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In the previous paper¹⁾, it was reported that as a result of the investigation of the alkaloids present in the domestic *Mahonia japonica* DC. (Japanese name "Hiiraginanten") the authors identified isotetrandrine and berbamine, which belong to the biscoclaurine type alkaloids, as tertiary bases, and jatrorrhizine, berberine and palmatine, as quaternary bases. The authors also clarified that in comparison with the alkaloids of the Indian *Mahonia*, studied by Chatterjee, et al.²⁾, the Japanese differed from the Indian in that in many cases the latter furnishes oxyacanthine as the biscoclaurine base, whereas the former contains isotetrandrine instead.

Now the authors have examined the alkaloids of the domestic *Mahonia Fortunei* Fedde (Japanese name "Hosoba-hiiraginanten"), and the results thereby obtained are reported in the following.

The plant material was obtained from the garden of Dr. K. Kimura, Professor of the Osaka University, where it grows wild, by his courtesy, and the alcoholic extract of the trunk was prepared as usual. The process for isolation of the alkaloids was quite the same as that described in the previous paper¹⁾. A comparison of the alkaloids contained in the Japanese and the Indian *Mahonias* is summarized in Table I.

As shown in Table I, the Japanese *Mahonia Fortunei* furnishes quite the same alkaloids, oxyacanthine and berbamine as the biscoclaurine bases, as the Indian *Mahonia*. In this connection, it is interesting to note that in Japan only two species, *Mahonia japonica* and *Mahonia Fortunei* grow wild, and of these two species, one furnishes isotetrandrine, and the other oxyacanthine. Besides, the fact gives a great suggestion

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