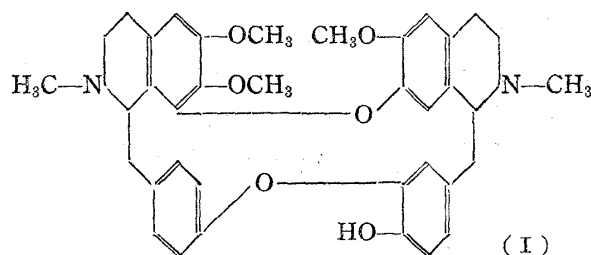


19. Masao Tomita,* Yasuo Inubushi,* and Eiichi Fujita** : Studies on the Alkaloids of Menispermaceous Plants. CXXIII.¹⁾ Paper Chromatography of Oxyacanthine and Repandine.

(Pharmaceutical Institute, Medical Faculty, University of Kyoto,* and Pharmaceutical Faculty, University of Tokushima**)

Repandine is an alkaloid which was first isolated by Bick and Whalley²⁾ from Australian *Daphnandra repandula* Banc. (family Monimiaceae) and which was later established by Bick and Todd³⁾ to have the same structure as and an optical isomer of oxyacanthine (I). The same workers further clarified that repandine was quite identical with the base previously obtained by Bruchhausen and Scholtze⁴⁾ by treatment of oxyacanthine (1 mole) with hydrogen chloride (1 mole). On the other hand, Bruchhausen⁵⁾ pointed out that repandine isolated by them did not originate naturally in plants but must presumably be an artefact produced from oxyacanthine by undergoing Walden inversion during the isolation process. Similar reaction in which oxyacanthine was allowed to react with $\frac{1}{2}$ equivalent of hydrogen chloride was followed by Taylor,⁶⁾



who stated that since repandine could not be obtained, the sample of oxyacanthine used by Bruchhausen *et al.* might not be a pure one and possibly contaminated with repandine from the beginning. However, this statement does not seem applicable for a series of our investigations on oxyacanthine isolated from Japanese *Berberis Thunbergii* DC., including the fission process of oxyacanthine⁷⁾ and the O-methyl ether⁸⁾ of repandine prepared from oxyacanthine by Bruchhausen's procedure, with sodium in liquid ammonia, and the cyclization reaction⁹⁾ of oxyacanthine with hydrobromic acid. Indeed, in these series of reactions, we could obtain no experimental results suggesting that a sample of oxyacanthine used had originally been accompanied with repandine.

So far as we have experienced, the most pure oxyacanthine was obtained as colorless pillars, m.p. 217°, but usually the sample in this state of purity could not be readily obtained by recrystallization. Consequently, the material used by us for the above miscellaneous reactions was somewhat less pure one of colorless pillars, melting at 210~213° or 214~216° (in an evacuated capillary),¹⁰⁾ $[\alpha]_D^{25} : +276^\circ$ (CHCl₃).

* Yoshida-konoe-cho, Sakyo-ku, Kyoto (富田真雄, 犬伏康夫, 藤田栄一).

** Minamijohzanjima-cho, Tokushima.

1) Part CXXII. M. Tomita, Y. Inubushi : This Bulletin, **3**, 7(1955).

2) I. R. C. Bick, T. G. Whalley : C. A., **41**, 1390(1947).

3) I. R. C. Bick, A. R. Todd : J. Chem. Soc., **1948**, 2170.

4) F. v. Bruchhausen, H. Scholtze : Arch. Pharm., **267**, 617(1929).

5) F. v. Bruchhausen : *Ibid.*, **283**, 44(1950).

6) W. I. Taylor : Private communication; R. H. F. Manske, H. L. Holmes : "The Alkaloids," IV, 218(1954), Academic Press Inc., New York.

7) E. Fujita : J. Pharm. Soc. Japan, **72**, 213, 217(1952).

8) E. Fujita, T. Saijoh : *Ibid.*, **72**, 1232(1952).

9) Y. Inubushi, K. Kozuka : This Bulletin, **2**, 215(1954).

10) Melting points are uncorrected.

Another information was also provided by us that in the preparation of repandine from oxyacanthine by Bruchhausen's method, even when the same sample was employed, in some case, repandine could be readily obtained, and in other case, could not in the least.

Under these circumstances, it has become necessary to reinvestigate the homogeneity and purity of a sample of oxyacanthine showing the same m.p. as that employed as the material of these reactions, and the condition under which repandine may form from oxyacanthine by the action of $\frac{1}{2}$ equivalent of hydrogen chloride.

First, the purity of a sample of oxyacanthine having m.p. 210~213° so far employed for our reactions was examined. As described in the experimental section, the contamination with repandine caused appreciable variations in the values of the specific rotation, and the Rf values in the paper chromatogram, of the material oxyacanthine. From these facts, the homogeneity of our sample of oxyacanthine was unequivocally established.

Since in our previous preparative work of repandine from oxyacanthine by Bruchhausen-Scholtze's⁴⁾ and our prescription,⁸⁾ in some case, repandine was obtained and not in others, similar technique was followed with the sample of oxyacanthine furnishing evidence for homogeneity in order to clarify this situation. As a result, it was found that the formation of repandine depended upon the amount of hydrochloric acid used, and the action of 1.12 moles of hydrogen chloride upon 1 mole of oxyacanthine invariably furnished the desired repandine.

We wish to express our appreciation to Dr. W. I. Taylor for his gift of a sample of oxyacanthine and to Mr. M. Kozuka for his help in this experiment. Our thanks are also due the Ministry of Education for financial aid.

Experimental¹⁰⁾

Oxyacanthine—Oxyacanthine purified through the sulfate from the methanolic extract of the stems of *Berberis Thunbergii* DC. was obtained as colorless pillars, m.p. 210~213° or 214~216°(in vacuum), and the m.p. was not raised any further after repeated recrystallization from a mixture of acetone and ether. $[\alpha]_D^{25}$: +276.1°(in CHCl_3 , $l=0.947$ dm., $c=1.035$). Also the sample of oxyacanthine¹¹⁾ provided by Taylor had nearly the same m.p. of 206~210° as ours, and the two materials showed no depression in m.p. on admixture. The most pure oxyacanthine¹²⁾ obtained by one of us (Tomita) from *Berberis Thunbergii* DC. showed m.p. 217°, $[\alpha]_D^{20}$: +279°(CHCl_3). On the other hand, repandine had m.p. 250~253° and $[\alpha]_D^{15}$: -106°(CHCl_3). When oxyacanthine was accompanied with repandine, the specific rotation of the mixture showed smaller value, as follows: A solution of 6.954 mg. of oxyacanthine (m.p. 210~213°) and 3.113 mg. of repandine in 1 cc. of CHCl_3 gave $[\alpha]_D^{25}$: +166°($l=0.5$ dm.). Oxyacanthine mixed with repandine melted between 208° and 213°, which apparently resembles that of impure oxyacanthine.

Paper Chromatography of Oxyacanthine and Repandine—The samples of oxyacanthine showing m.p. 217° and m.p. 210~213°, and their mixture with repandine were chromatographed on Toyo Roshi No. 50, and the potassium salt of tetrabromophenolphthalein ethyl ester was used for the detection of alkaloidal spots. The procedure was the same as that described by Bick and Todd.¹³⁾ As shown in Table I, when oxyacanthine was mixed with repandine, a somewhat broad spot appeared in the middle of their respective spots. Attempts to separate it into two spots by varying the solvent and the mixing ratio of the solvents failed.

TABLE I.

Substance	Rf in BuOH:AcOH:H ₂ O =63:10:27 (cc.)	Rf in BuOH:AcOH:H ₂ O =25:0.5:25 (cc.)
Oxyacanthine(m.p. 217°)	0.70	0.49
Oxyacanthine(m.p. 210~213°)	0.70	0.49
Oxyacanthine(Späth) (m.p. 206~210°)	—	0.49

11) This sample was supplied Dr. W. I. Taylor by the late Dr. E. Späth.

12) H. Kondo, M. Tomita: Arch. Pharm., **268**, 1(1930).

13) I. R. C. Bick, A. R. Todd: J. Chem. Soc., **1949**, 2767.

Repandine(m.p. 250~253°)	0.74	0.61
Repandine + Oxyacanthine(m.p. 217°)	0.73	0.55
Repandine + Oxyacanthine(m.p. 210~213°)	0.73	0.55

Conditions under which Repandine may form from Oxyacanthine—A procedure essentially similar to that described in the previous paper⁸⁾ was followed. In this experiment, the amount of 0.1 *N* HCl used was strictly defined. As is apparent from Table II, the action of the amount of 0.1 *N* HCl equivalent to 1.12 moles of HCl upon 1 mole of oxyacanthine invariably gave repandine.

TABLE II.

Oxyacanthine m.p. 210~213° (mg.)	Amount of 0.1 <i>N</i> HCl (mole)	Standing at room temp. (hrs.)	Yield of repandine (mg.)	Recovered oxyacanthine (mg.)
500	1.00	36	—	300 ^{a)}
500	1.00	48	—	300 ^{a)}
500	0.96	48	—	300 ^{a)}
300	1.02	48	—	140 ^{a)}
300	1.20	48	—	130 ^{a)}
300	0.74	48	—	150 ^{a)}
300	0.92	48	—	140 ^{a)}
300	1.12	48	63	— ^{b)}
500	1.12	48	120	— ^{b)}
500	1.12	48	110	— ^{b)}

a) The filtrate from recovered oxyacanthine left a viscous oily product.

b) The residue left after separation of repandine gave a viscous oil, from which oxyacanthine could not be recovered.

Summary

The purity and homogeneity of the sample of oxyacanthine showing m.p. 210~213° or 214~216° (in vacuum), $[\alpha]_D^{25} : +276.1^\circ$ (CHCl_3), employed in our previous work was examined by paper chromatography, etc. These techniques afforded no evidence that it was accompanied with its optical isomer, repandine.

The conditions under which repandine may form from oxyacanthine by treatment with hydrogen chloride was also investigated using the same sample of oxyacanthine but furnished no evidence for its contamination with repandine. As a result, it was established that the action of 1.12 moles of hydrogen chloride upon 1 mole of oxyacanthine invariably gave repandine.

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