56. **Shigehiko Sugasawa, Takashi Tatsuno, and Takashi Kamiya:** Synthesis in the Benzoquinolizine Group. XXI[1]. Synthesis of Thiazolyl- and Quinolyl-benzoquinolizine Derivatives.

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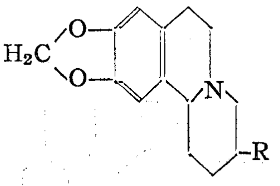
In the previous paper of this series[2], we reported on the synthesis of 4′,5′-methylenedioxy-3,4,5,6,7,8-hexahydro-6-(N-methylpyrrolidyl-2″)-(2′,1′ : 1,2-benzoquinolizine) (A) by our general method starting from *l*-nicotine and its constitution was established beyond doubt. The present paper deals with the synthesis of similar compounds having 2-methylthiazolyl-(4)- and 6,7-methylenedioxyquinolyl-(2)- group attached to the same benzoquinolizine nucleus at 6-position, i.e., 4′,5′-methylenedioxy-3,4,5,6,7,8-hexahydro-6-(2″-methylthiazolyl-4″)- and -6-(6″,7″-methylenedioxyquinolyl-2″)-(2′,1′ : 1,2-benzoquinolizine) (B and C), in order to examine how the amoebicidal property of basic benzoquinolizines is influenced by the change in the basic side chain.



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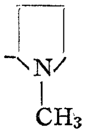

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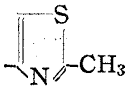

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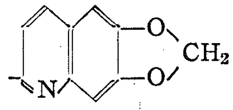

Both of these compounds were prepared from 3-acetylpyridine (I), which was brominated with bromine in hydrobromic acid, furnishing 3-ω-bromoacetylpyridine (II). This was then condensed with thioacetamide in the presence of potassium acetate, giving 3-(2′-methylthiazolyl-4′)-pyridine (III).

On the other hand, (I) was condensed with 6-aminopiperonal in alcohol by means of potassium hydroxide and 3-(6′,7′-methylenedioxyquinolyl-2′)-pyridine (IV) was thus obtained in a fair yield.

β-3,4-Methylenedioxyphenethyl bromide was now heated with (III) and (IV), respectively, forming the corresponding quaternary salts (Va) and (Vb). It was found neccessary to heat in boiling xylene to obtain (Vb), whereas benzene was a suitable solvent for the preparation of the former. Some differences in the properties were also observed between (Va) and (Vb) as is mentioned in the experimental section. In both cases pyridine obviously offers the preferred basic center to form the quaternary salts.

The oxidation of (Va) and (Vb) by means of potassium ferricyanide under appropriate working conditions (cf. Experimental Section) yielded the corresponding pyridones (VIa) and (VIb). As for the position occupied by the carbonyl group in both pyridones, we propose fromulae (VIa) and (VIb) rather than their alternatives in view of the result obtained in the oxidation of pyrrolidyl analog (v. s.). Values of their dipole moments as were measured by one of us (cf. the forth comming paper by Tatsuno) seems to provide futher support of our view.

The pyridones were then ring-closed by boiling with phosphoryl chloride, yielding the benzoquinolizinium salts (VIIa) and (VIIb), which were characterized as their crystalline iodides. When treated with aqueous alkali hydroxide these quinolizinium salts undergo smooth ring fission, giving the original pyridones back in good yields, which behavior has

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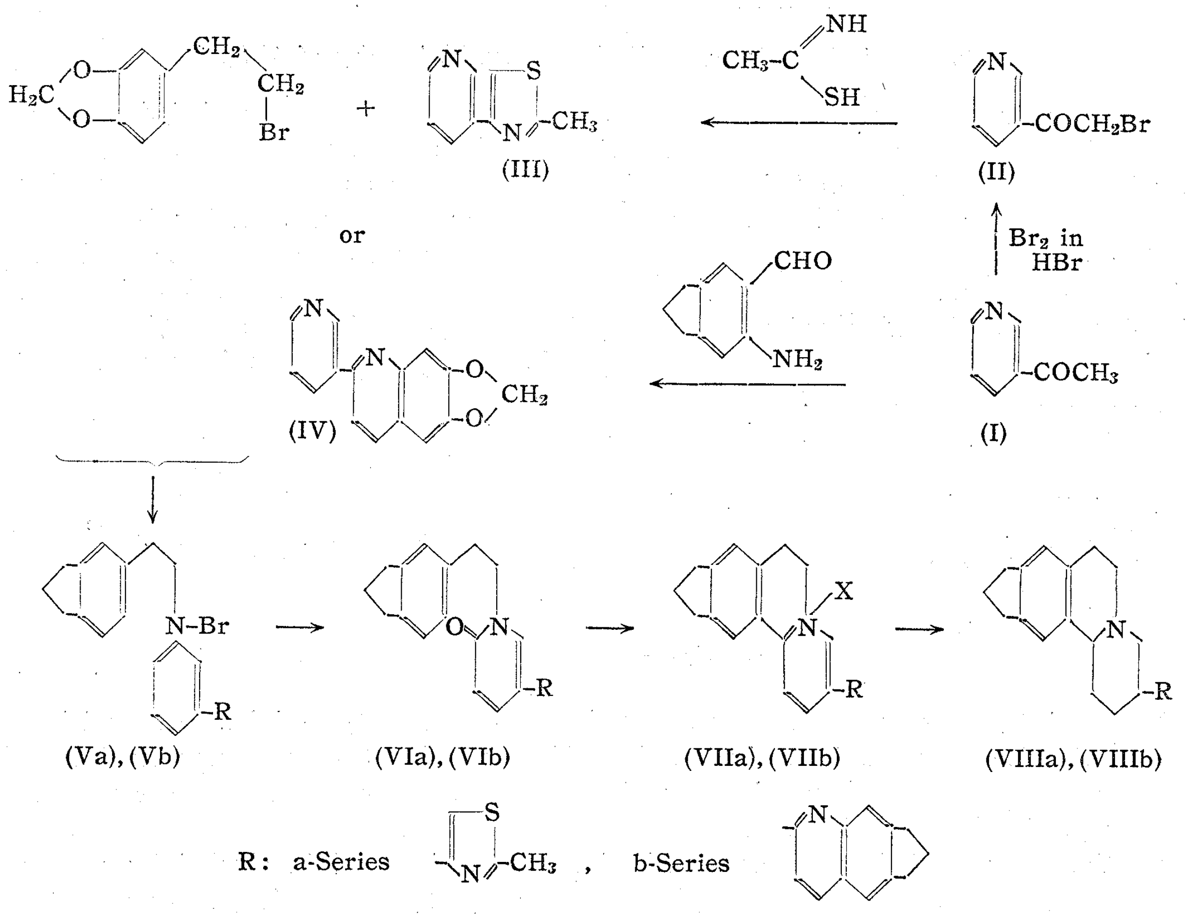
[1] S. Sugasawa, K. Oka: This Bulletin, 1, 230 (1953).

[2] J. Pharm. Soc. Japan, 72, 248(1952).

already been observed by one of us (S.S.) and Akahoshi[3].

The reduction of (VIIa) and (VIIb) was carried out as usual and the tertiary bases (VIIIa=B) and (VIIIb=C) were produced in good yields.



The authors' thanks are due to Messrs. Hung, Sakai, Kaneko, Fukuda, and Sekijima and Mrs. Wu for microanalytical data.

Experimental

I. Synthesis of 4′,5′-methylenedioxy-3,4,5,6,7,8-hexahydro-6-(2″-methylthiazolyl-4″)-(2′,1′:1,2-benzoquinolizine) (VIIIa=B)

3-Acetylpyridine (I)—Prepared according to Kolloff and Hunter[4]. Colorless liquid of $b.p_{10}$ 115°; yield, 84%.

3-ω-Bromoacetylpyridine (II)—The hydrobromide of (II) was prepared by Dornow's method[5]. Yield, 90% of a crude substance of m.p. 190~193°. Pure hydrobromide (from water) melts at 196~198° (Dornow gives 198°).

3-(2′-Methylthiazolyl-4′)-pyridine (III)—The foregoing bromoketone hydrobromide (5.2 g.) and thioacetamide (1.5 g.) were dissolved in alcohol, and to this mixture an aqueous solution of potassium acetate (8 g. in 20 cc. of water) was added dropwise with stirring while being warmed on a water bath. After being boiled for 4 hrs. alcohol was removed in vac. and the residue was added with water, extracted with ether, dried, and evaporated. The product distilled at 160~165°/10 mm. and solidified on standing. Formed colorless needles of m.p. 82° from benzine-ligroine. *Anal.* Calcd. for $C_9H_8N_2S$: N, 15.9. Found: N, 15.7.

3-(2′-Methylthiazolyl-4′)-N-β-3,4-methylenedioxyphenethylpyridinium bromide (Va)—The above-mentioned compound (1.0 g.), β-3,4-methylenedioxyphenethyl bromide (1.3 g.), and benzene (5 cc.) were refluxed on a water bath, giving a clear solution, from which needle-shaped crystals began to separate out after ca. 20 min. After being boiled about an hour crystalline solid separated

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3) S. Sugasawa, S. Akahoshi: J. Pharm. Soc. Japan, 72, 1273(1952); Akahoshi: *Ibid.*, 72, 1277(1952).
4) Kolloff, Hunter: J. Am. Chem. Soc., 63, 490(1941).
5) Ber., 84, 148(1951).

was filtered and the filtrate was again heated, furnishing some more crystals. This procedure was repeated twice more and 1.7 g. of the product (Va) was collected altogether. Sparingly soluble in cold water and purified from hot water, giving yellowish needles of m.p. 250∼251° (decomp.). *Anal.* Calcd. for $C_{18}H_{17}O_2N_2SBr$: N, 6.9; Br, 19.7. Found: N, 7.0; Br, 20.2.

N-(β-3′,4′-Methylenedioxyphenethyl)-5-(2″-methylthiazolyl-4″)-pyridone-2 (VIa)—The above-mentioned bromide (2 g.) in water (300 cc.) was mixed dropwise with aqueous sodium hydroxide (15 g. in 500 cc. of water) at 0°. After adding 100 cc. of benzene, the mixture was added with potassium ferricyanide (2 g.) in a minimum amount of cold water at 6°. Then the whole was stirred for about 2 hrs. at 15∼20°. The aqueous layer, which was separated from the benzene layer, was extracted with benzene and both benzene solutions were combined, washed, dried, and evaporated, leaving a brown solid (1.2 g.), which was again dissolved in dry benzene and filtered through an alumina column. The residue of the benzene eluate was purified from the same solvent, forming colorless aggregated needles of m.p. 152∼153°. *Anal.* Calcd. for $C_{18}H_{16}O_3N_2S\cdot 1/2H_2O$: C, 61.85; H, 4.5; N, 8.0. Found (in substance dried at 60° in vac.): C, 62.5; H, 4.7; N, 7.8.

4′,5′-Methylenedioxy-3,4-dihydro-9,10-dehydro-6-(2″-methylthiazolyl-4″)-(2′,1′:1,2-benzoquinolizinium) salt (VIIa)—The afore-mentioned pyridone (0.5 g.) and phosphoryl chloride (5 cc.) were boiled gently in an oil bath for 1 hr. After evaporating excess of phosphoryl chloride the residue was treated with ice water, separating white solid, which was dissolved in warm water, filtered, and the filtrate was neutralized with sodium bicarbonate. The solution was extracted with benzene and the aqueous layer, after filtered through a wet filter, was added with sodium iodide, giving a faint yellow amorphous iodide (VIIa: X=I). Yield, 4.4 g. of m.p. 190∼193° (decomp.).

4′,5′-Methylenedioxy-3,4,5,6,7,8-hexahydro-6-(2″-methylthiazolyl-4″)-(2′,1′:1,2-benzoquinolizine) (VIIIa)—The above-mentioned iodide (0.23 g.) was converted into the corresponding chloride by the usual method and was reduced catalytically over Adams' Pt catalyst at about 40°, 3 moles of hydrogen being absorbed smoothly, and the product was worked up as usual, giving crude (VIIIa). This was purified from alcohol-benzine, forming colorless needles of m.p. 167∼169° (decomp.). Yield, 0.12 g. *Anal.* Calcd. for $C_{18}H_{20}O_2N_2S\cdot HCl$: C, 59.1; H, 5.5; N, 7.6. Found: C, 59.3; H, 5.7; N 7.3.

II. 4′,5′-Methylenedioxy-3,4,5,6,7,8-hexahydro-6-(6″,7″-methylenedioxyquinolyl-2″)-(2′,1′:1,2-benzoquinolizine)

6,7-Methylenedioxy-3-(β-pyridyl)-quinoline (IV)—A mixture of 3-acetylpyridine (3.5 g.), 6-aminopiperonal (5 g.), and alcoholic potassium hydroxide solution (40 cc. of 1%) was warmed on a water bath, giving reddish clear solution. After being refluxed gently for 30 min. alcohol was evaporated and the residue was mixed with cold water, furnishing white crystalline solid, which was filtered, washed, and dried. Purified from acetic ester, forming colorless needles of m.p. 154∼155°. Yield 6∼6.2 g. or 65∼67%. *Anal.* Calcd. for $C_{15}H_{10}O_2N_2$: C, 72.0; H, 4.0; N, 11.2. Found: C, 72.0; H, 4.0; N, 11.0.

3-(6″,7″-Methylenedioxyquinolyl-2″)-N-β-(3′,4′-methylenedioxyphenethyl)-pyridinium bromide (Vb)—A mixture of the foregoing pyridylquinoline (0.6 g.), β-3,4-methylenedioxyphenethyl bromide (0.6 g.), and pure xylene (7 cc.) was heated in an oil bath (130∼135°), separating gradually the quaternary bromide on the wall. This was filtered and the filtrate was again warmed as above, giving the second crop. This procedure was repeated four times altogether, yielding the addition product (Vb) in nearly quantitative yield. Purified from boiling water (Vb) formed fluffy crystals of m.p. 120°. For analysis this was dried first at 80° and then at 100°, the m.p. was thus raised to 232∼233°, which still holds water of crystallisation as is shown by the following analytical data. *Anal.* Calcd. for $C_{24}H_{19}O_4N_2Br\cdot 2H_2O$: C, 54.0; H, 4.5; N, 5.4. Found: C, 54.2; H, 4.5; N, 5.8.

N-(β-3′,4′-Methylenedioxyphenethyl)-5-(6″,7″-methylenedioxyquinolyl-2″)-pyridone-2 (VIb)—The foregoing compound (Vb) (2 g.) in water was thoroughly kneaded with fresh silver oxide, prepared from 5 g. of silver nitrate. The filtrate from silver bromide and silver oxide, together with washing water, was mixed with a conc. solution of potassium ferricyanide (3 g.), separating yellow solid substance. To this mixture was now added sodium hydroxide pellets (30 g.) below 15° and then the whole was stirred for 2 hrs. at 20°. The pyridone (VIb) thus formed was now taken up in benzene, washed, dried, and purified through an alumina column, and evaporated. The residue was recrystallized from benzene, forming faint yellow needles of m.p. 193∼194°, yield, 0.9 g. of pure substance. *Anal.* Calcd. for $C_{24}H_{18}O_5N_2$: C, 69.5; H, 4.4; N, 6.8. Found: C, 69.4; H, 4.3; N, 7.1.

4′,5′-Methylenedioxy-3,4,5,6,7,8-hexahydro-6-(6″,7″-methylenedioxyquinolyl-2″)-(2′,1′:1,2-benzoquinolizine) (VIIIb)—The pyridone (VIb) (0.4 g.) was mixed with phosphoryl chloride (12 cc.) and the mixture was refluxed gently in an oil bath for 2 hrs. Excess of phosphoryl chloride was removed in vac., and the residue was treated with ice water, separating white crystalline solid. This was purified from hot water, giving pure quinolizinium chloride (VIIb: X=Cl), which was directly used for the next step. Crystalline iodide and picrate (yellow needles of m.p. 260° (decomp.)) were also prepared.

The chloride (0.35 g.) in pure acetic acid (50 cc.) was reduced catalytically over Adams' Pt catalyst, three moles of hydrogen being absorbed smoothly. The product was worked up as usual, giving the hydrochloride of the base (VIIIb). Purification of the hydrochloride from various solvents so far tried was not successful and it was best obtained by adding benzene-hydrogen chloride to the dried benzene solution of the free base. The hydrochloride thus prepared gives turbid solution when dissolved in water, which is clarified by adding aqueous hydrochloric acid, showing that basicity of the base (VIIIb) is fairly weak. Characterized as crystalline picrolonate, which forms yellow needles of m.p. 157～158°(decomp.) from alcohol. *Anal.* Calcd. for $C_{34}H_{30}O_9N_6\cdot 2H_2O$: C, 58.1; H, 4.9; N, 12.0. Found (in substance dried at 60° in vac.): C, 57.8; H, 4.9; N, 12.2.

Summary

Two new basic hexahydrobenzoquinolizines, 6-thiazolyl and 6-quinolyl derivatives, were prepared. Their amoebicidal properties will be examined.

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57. **Yosoji Ito, Bun-ichi Tamaoki, and Hideharu Sakamoto:** Studies on Insulin Assay. I.

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While the rabbit method of insulin assay has been widely adopted by the Pharmacopoeia in Japan and other countries, the mouse method of the same has been playing an important part in fundamental research as a routine assay method.

As the mouse method is based on all-or-none type or quantal response, the scientifically standardized analysis for this assay could not be found until the probit method was well established and introduced in the field of bioassay[1~4]. In other words, without the metameter by which the sigmoid curve of log dose-percentage relationship can be mathematically transformed into a straight line, the statistical analysis could not be efficiently applied to this type of biological assay, and therefore, the experimental results would remain only descriptive and of no more information.

It is the purpose of this paper to discuss this quantal assay method of insulin, using the modern statistical procedure such as the probit analysis.[4]

The expense for the work on this mouse assay method of insulin was partly defrayed by the Scientific Research Fund for the years of 1952 and 1953 from the Ministry of Education, to which the authors' thanks are due. The authors are also grateful to Mr. T. Morioka who kindly helped them in this work.

Experimental Methods and Materials[5]

1) **Experimental Animal**—Common white mice weighing between 25～30 g. were used. Male and female mice were separately raised in rooms of a temperature range between 10～17° and, before the test, both animals were pooled and then were divided at random into the corresponding groups for the purposes. 2) **Colony Diet**—Animals received wheat, barley, broken rice, dried fish-meat, and green vegetable, *ad libitum*. 3) **Fasting Periods**—Before the injection of insulin, animals were fasted for 2 hours. 4) **Treatment of Mice during the Tests**—After injection of insulin, animal was transferred into the incubator with air vents which was thermostatically controlled at 30±0.5°. 5) **Determination of the Animal Response**—Most mice which reacted to insulin showed a very remarkable convulsion after their activities were weakened. However, there were found some proportion of mice which showed a state of collapse determined by the failure of the animals to right itself when placed on its back. According to our experience, this

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1) C.I. Bliss: Ann. Appl. Biol., **22**, 134, 307(1935); *ibid.*, **24**, 815 (1937).

2) C.I. Bliss; Quart. J. Pharm. & Pharmacol., **11**, 192 (1938).

3) D.J. Finney: Suppl. J. Roy. Stat. Soc., **9**, 46 (1947).

4) D.J. Finney: "Probit Analysis", 2nd Ed., Cambridge University Press, England (1952).

5) K.L. Smith: "Hormone Assay", Ed. C.W. Emmens, Chap. II, Academic Press, U.S.A. (1950).