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32. Masazumi Kawanishi: Synthesis of the So-called Dehydro-rotundinium Salt and Its Partial Reduction.

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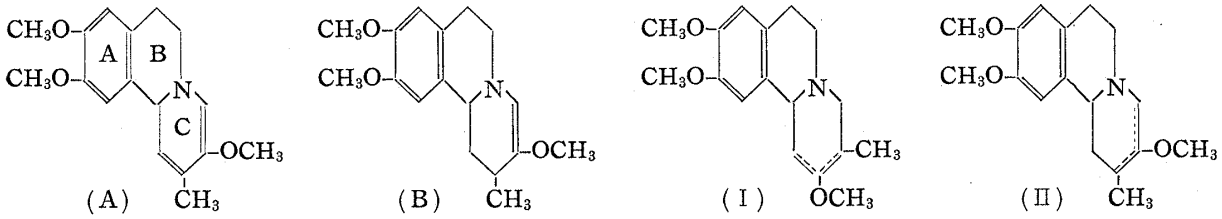
In 1944, H. Kondo and Matsuno[1] isolated a new alkaloid, $C_{16}H_{21}O_3N$, from the rhizome of *Stephania rotunda* LOUREIO, indigenous to French Indo-China. They named this base rotundine and Matsuno proposed the structure (A) for this base and (B) for dihydro-rotundine, the reduction product of rotundine.



Recently, for the purpose of providing synthetical support for these structures, Sugasawa and Mizukami[2] synthesized the compound (I), the position isomer of (B), and (II), which they tentatively called *rac*-dihydro-isorotundine and *rac*-dihydro-rotundine, respectively, but they could not draw conclusion about the validity of (A) and (B). Later, a synthesis of *rac*-tetrahydro-rotundine was described by Kametani and Nomura.[3]

Succeeding the work of Sugasawa and Mizukami[2] and for the same purpose, the present writer studied the dehydrogenation reaction of the compound (II) and partial hydrogenation of the product. Dihydro-rotundine, prepared from the natural *l*-rotundine,[*2] was treated similarly, and it was finally concluded that the structure (A) cannot represent rotundine.

Prior to the experiment with (II), some model experiments were carried out with the more readily accessible (I). Mercuric acetate was used as the dehydrogenation agent, which was successfully applied by Schnider, *et al.*[4] in their recent work on the C-ring

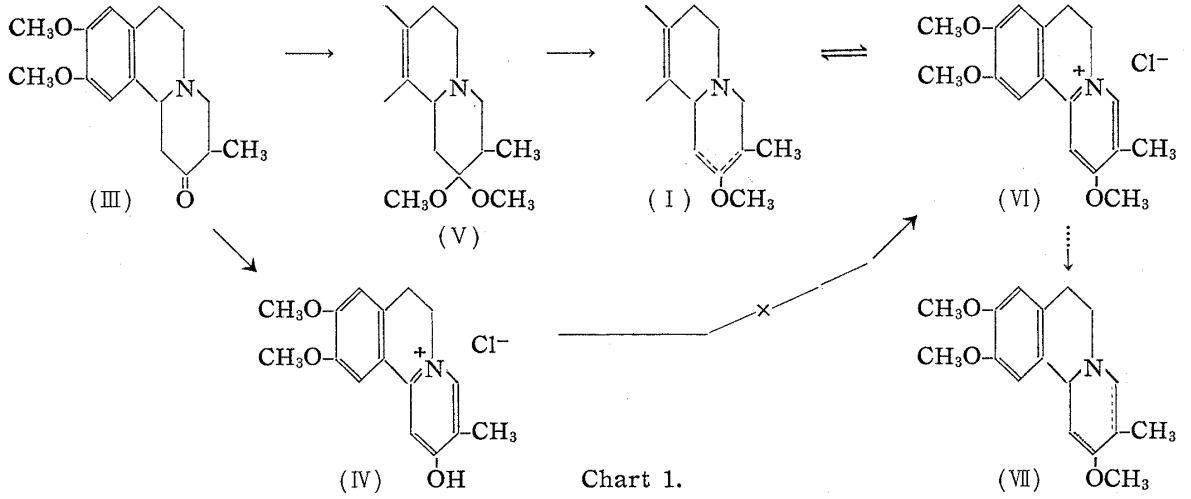


Chart 1.

[*1] Toda-machi, Kita-adachi-gun, Saitama-ken (川西正純).

[*2] Grateful acknowledgement is offered to Prof. H. Kondo for the gift of *l*-rotundine.

1) H. Kondo, T. Matsuno: Yakugaku Zasshi, **64B**, 113 (1944).
2) S. Sugasawa, K. Mizukami: This Bulletin, **6**, 313, 359 (1958).
3) T. Kametani, Y. Nomura: *Ibid.*, 8, 685 (1960).
4) A. Brossi, H. Lindler, M. Walter, O. Schnider: Helv. Chim. Acta, **41**, 131 (1958).

aromatisation of 3-ethyl-9,10-dimethoxy-1, 2, 3, 4, 6, 7-hexahydro-11b *H*-benzo[*a*]quinolizin-2-one. Thus, as is shown in Chart 1, the ketone (III), dissolved in 10% acetic acid, was treated with mercuric acetate and a stable dehydrogenation product was isolated as a quaternary chloride (IV), whose m.p. 267~268° (decomp.) and ultraviolet data were in good agreement with those mentioned by Schnider for this compound. O-Methylation of this substance was attempted by the agency of diazomethane, dimethyl sulfate, and methanol-hydrogen chloride, but uniformly without effect, recovering the starting material.

Dehydrogenation of *rac*-dihydro-isorotundine (I) prepared from the dimethyl ketal (V) of the ketone (III) according to the method of Mizukami,[2] was therefore investigated. Mercuric acetate in hot 10% acetic acid gave an unsatisfactory result, yielding a considerable amount of a phenolic quaternary salt (IV) as a by-product. This result is probably due to hydrolysis of the hemiketal group of (I) to produce the ketone (III), by not dilute acid which underwent dehydrogenation by mercuric acetate to yield (IV). Therefore, the dehydrogenation was attempted in methanolic solution at 60~70°, when 3-methyl-2,9,10-trimethoxy-6,7-dihydrobenzo[*a*]quinolizinium salt (VI) was produced as a rather stable compound in a fair yield.

In order to effect a selective hydrogenation of $-\overset{|}{C}=N-$ bond in (VI), the methanolic solution of this compound was treated under a variety of working conditions with as much of sodium borohydride as to generate one molar equivalent of hydrogen. However, the product was always the tetrahydro derivative, recovering the starting material on the other hand. The reduction product was found to be identical with *rac*-dihydro-isorotundine.

Awe, *et al.*[5] reported that when berberine-type quaternary salt was reduced with sodium borohydride, either in ethanolic or methanolic solution, a tetrahydro derivative was produced, while $-\overset{|}{C}=N-$ group alone was reduced with even an excess of the same

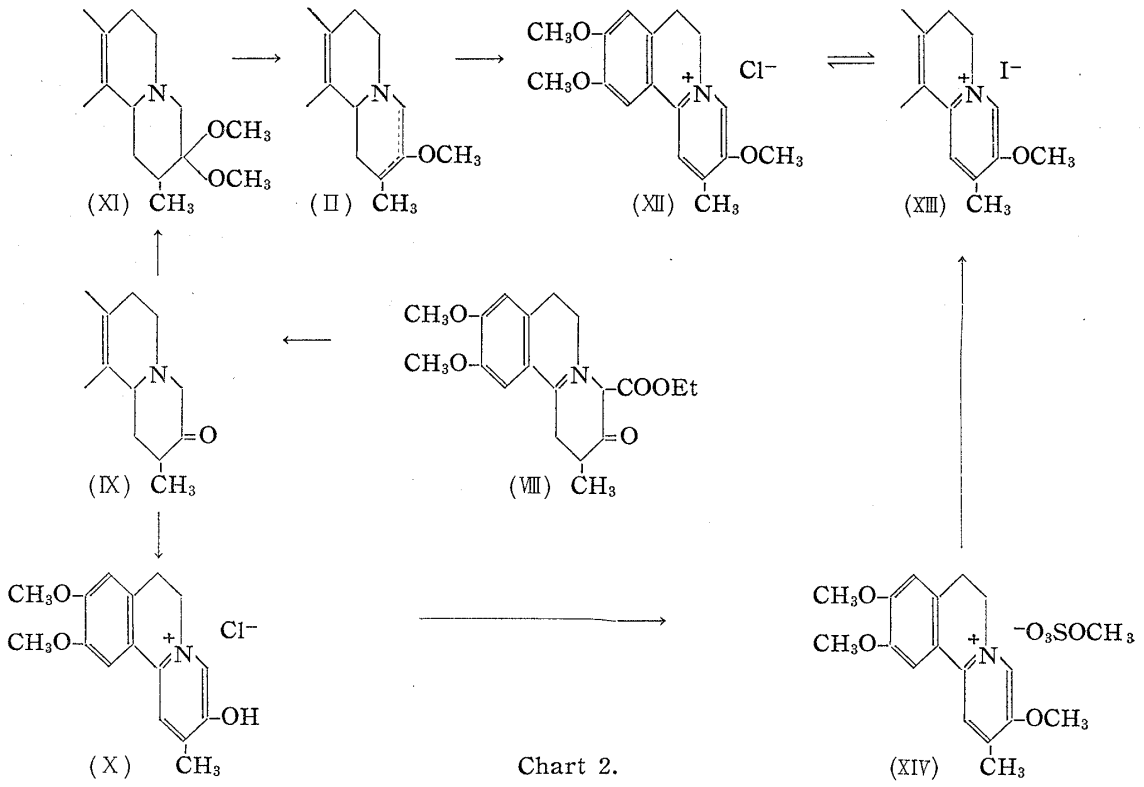


Chart 2.

5) W. Awe, H. Wichmann, R. Buerhop: Chem. Ber., **90**, 1997 (1957).

reagent if used in tetrahydrofuran solution. (VI) dissloved in tetrahydrofuran was therefore reduced with sodium borohydride during 24 hours. In the crude reduction product thus obtained, the presence of diene-like compound (VII) of rotundine (A)-type was surmised from inspection of its infrared and ultraviolet spectra, but this base was found too unstable to be purified. An attempt to prepare its methiodide was also unsuccessful, producing a mixture of *rac*-dihydro-isorotundine (I) methiodide and an unspecified, deeply colored substance. Partial reduction was impossible also with lithium aluminium hydride in ether or with sodium hyposulfate.

The experiment was next extended to *rac*-dihydro-rotundine (II). As shown in Chart 2, the rather unstable β-piperidone (IX) prepared from the keto-ester (VIII), was converted to dimethylketal derivative (XI), which was distilled in high vacuum according to the method of Mizukami. Assuming the distillate as the expected hemi-ketal, this was treated with mercuric acetate in methanolic solution as above. Rather unexpectedly, the product was found to contain a phenolic substance, which gave a positive color test with ferric chloride. This may be due to the fact that some of the ketal distilled over without suffering the loss of CH_3OH and hence the distillate was a mixture of the hemi-ketal and the original dimethyl ketal, the latter of which underwent hydrolysis by acetic acid liberated during the dehydrogenation reaction to yield the ketone (IX), which was further dehydrogenated by mercuric acetate to form the phenolic quaternary salt (X). Direct dehydrogenation of (IX) was, therfore, attempted with mercuric acetate in 10% acetic acid solution, when, as was expected, the phenolic quaternary salt (X) was obtained in a good yield. It may be worth mentioning that contrary to the 2-hydroxy compound, which is indifferent to ferric chloride, (X) gives a distinct positive color test with this reagent.

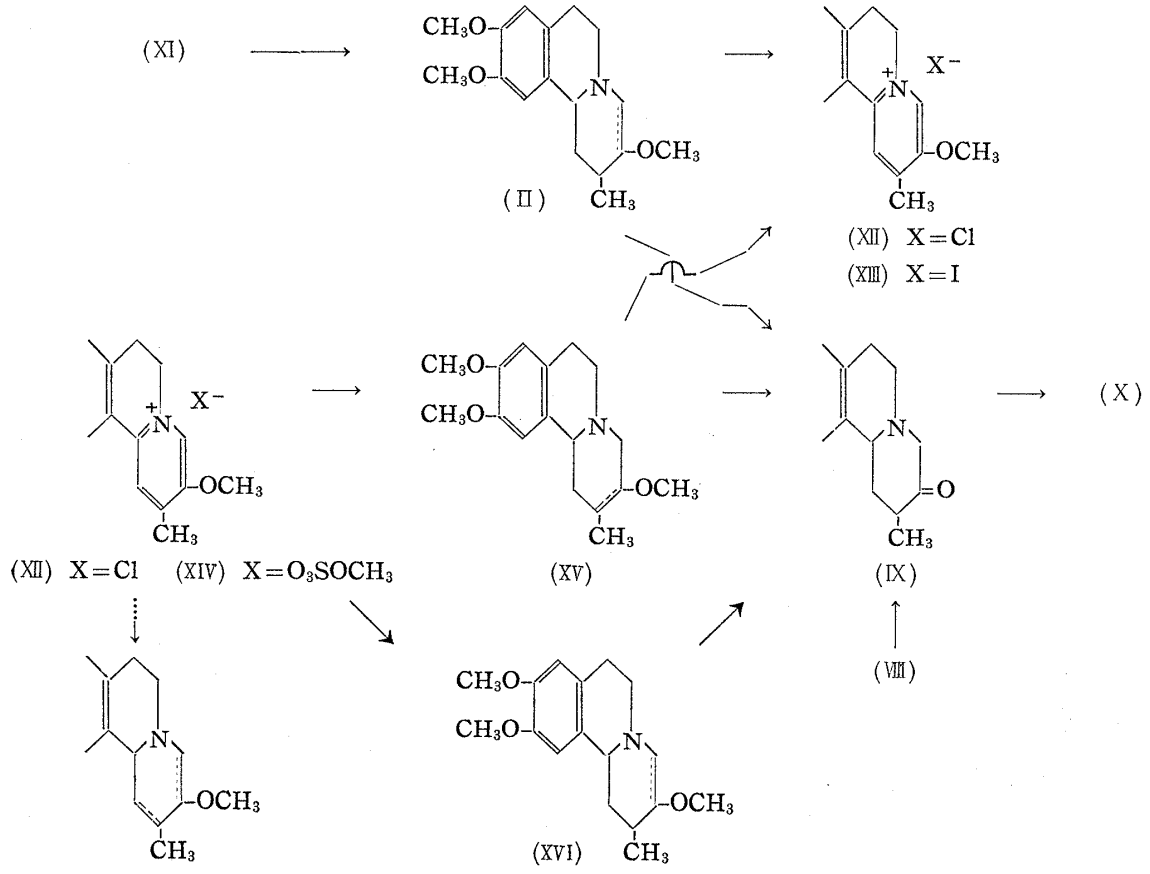


Chart 3.

Though O-methylation of (X) was unsuccessful with diazomethane, dimethyl sulfate, and methanolic hydrogen chloride, recovering the starting material, dimethyl sulfate in combination with an excess of 33% potassium hydroxide solution, as was successfully used by Robinson and Sugasawa[6] in O-methylation of dehydrolaudanosolinium salt, proved to be effective in giving the compound (XIV), which was converted to the corresponding chloride (XII) and iodide (XIII), all giving correct analyses as such. The same compound (XII) was also obtained by dehydrogenation of the pure hemi-ketal, prepared from the ketal (XI) by heating towards 200° with aluminium *tert*-butoxide prior to vacuum distillation. The compound (XII) prepared by both procedures formed faint yellow needles, m.p. 234~235° (decomp.), and their identity was proved by their superimposable infrared and ultraviolet spectra. As the method for preparation of (XII), the former is by far preferable to the latter, because reproducible result cannot be obtained with the latter method.

In case the proposed structure of rotundine were correct, its reduction product, dihydro-rotundine, should undergo a smooth dehydrogenation under the above-mentioned working conditions to give dehydro-rotundinium salt, which should be identical with the one prepared as above, after the sole asymmetric center present in the original compound is abolished.

Therefore, dihydro-rotundine prepared from natural rotundine was treated with mercuric acetate in methanolic solution as above and the product was isolated as the chloride in a good yield. When purified from methanol and diisopropyl ether mixture, this formed crystals of m.p. 203~204° (decomp.), which was raised to 205~206° by further purification from the same solvent mixture, forming yellow brown prisms. This was

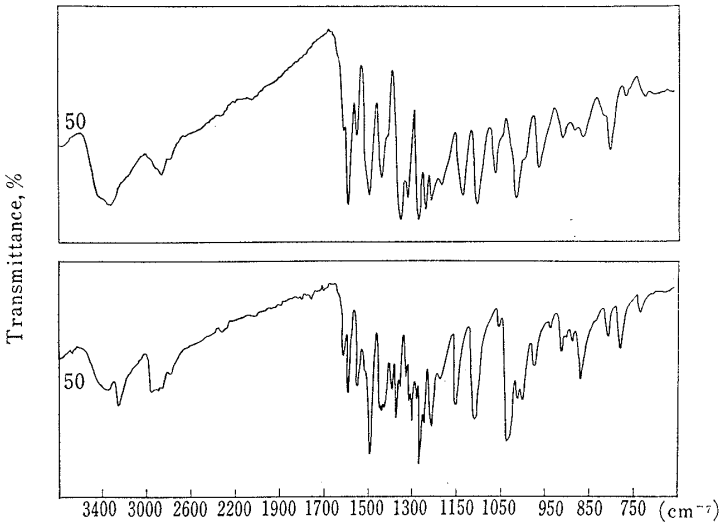


Fig. 1. Infrared Spectra (in KBr) (Koken Model DS-301)

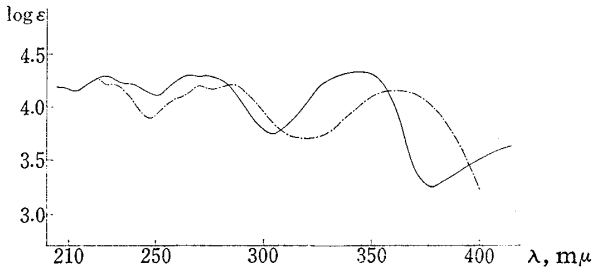


Fig. 2. Ultraviolet Spectra (in EtOH) (Carry Model 11)
——— Dehydrorotundinium chloride
-·-·- (XII)

6) R. Robinson, S. Sugasawa : J. Chem. Soc., **1932**, 789.

designated as natural dehydro-rotundinium salt to discriminate it from the synthetic product.

Though the ultraviolet data (Fig. 2) appeared to suggest the presence of a similar conjugated system in both substances, they could not be identical judged from their different color, decomposition points, and infrared data (Fig. 1), thus rendering the proposed structure of rotundine improbable.

In order to acquire some information about the possibility of the presence of such a diene system as is assumed in the C-ring of rotundine, partial reduction of synthetic dehydro-rotundinium salts (XII and XIV) was examined under a variety of working conditions. In one example using 4 molar equivalents of sodium borohydride to 1 molar equivalent of (XII) and (XIV), there was produced much tarry substance insoluble in ether, whose infrared spectrum suggested the presence of a compound having a diene system, but this product was very unstable and could not be characterized even as a methiodide. Reduction with sodium borohydride in various solvents or with lithium aluminium hydride under a variety of conditions was unsuccessful, showing the difficulty of preparing the diene system as was proposed in the C-ring of rotundine.

During the course of sodium borohydride reduction there was isolated a small amount of colorless prisms from the resinous reduction product, whose infrared data revealed the absence of a diene system. Since this appeared to be a dihydro-rotundine type of compound, the salt (XII) and (XIV) were reduced with sodium borohydride in methanolic solution and the product, dissolved in hexane, was purified through an alumina column and recrystallized from hexane, when a base of m.p. 98~99° was isolated in a fair yield, identical with the one mentioned above. Though the free base was fairy unstable in the air, coloring brownish and rendering its analysis impossible, its methiodide of m.p. 248~249° (decomp.) was quite stable to allow its characterisation. On admixture with the methiodide of *rac*-dihydro-rotundine of m.p. 247~248° (decomp.), there was a depression of 3~4°, suggesting non-identity of these two substances. Difference in their infrared spectra also lent support to this view; a band ascribed to the isloated double bond is absent while there is a weak but distinct band at 1710 cm^{-1} of inexplicable nature in the infrared spectrum of this new base (XV).

For the purpose of elucidating the structure of (XV), its methanolic solution was treated with mercuric acetate as usual and the product was isolated as the chloride and characterised as the iodide, which were found to be identical with the chloride and iodide of (XIII).

The base (XV), when treated with 10% hydrochloric acid, formed a ketonic substance, whose methiodide formed dices of m.p. 232~233°, identical with the authentic methiodide of the ketone (IX). By the agency of mercuric acetate, this ketone yielded 3-hydroxylated quaternary salt (X) identical with the authentic specimen. These facts lent strong support for the presence of $-\overset{|}{C}=\overset{|}{C}(OCH_3)$ group in the C-ring of (XV), in spite of the negative data against the presence of an isolated double bond in its infrared spectrum.

Catalytic hydrogenation of (XII) was now examined. Only in a faint alkaline solution, this absorbed 2 molar equivalents of hydrogen activated over the Adams platinum catalyst to give an oily base in a good yield. When the reduction was interrupted after 1 molar equivalent of hydrogen had been absorbed and the product was worked up, there was obtained a highly unstable base, which soon resinified to become insoluble in ether. From such a resinous material a small amount of a stable base was isolated through alumina chromatography and was found to be identical with the one obtained as above. Thus, it was again proved impossible to catalytically reduce the $-\overset{|}{C}=N-$ group selectively. In this base, which remained oily, the presence of an isolated double bond was assumed from its infrared absorption band at 1670 cm^{-1}, but its out-of-plane absorption was not

the same with that of *rac*-dihydro-rotundine (II). The fact that this base yielded the ketone (IX) when treated with dilute hydrochloric acid and that the methiodide gave good analyses as methiodide of (XVI) supported the presence of an enol methyl ether group in the C-ring. Acid hydrolysis of (II), which has hitherto been left unstudied, also yielded the same ketone (IX) with ease.

It is concluded that the compounds (II), (XV), and (XVI) are isomers with the same substituent groups in the same positions, each having one double bond in the C-ring. From their infrared data the following deduction may be drawn.

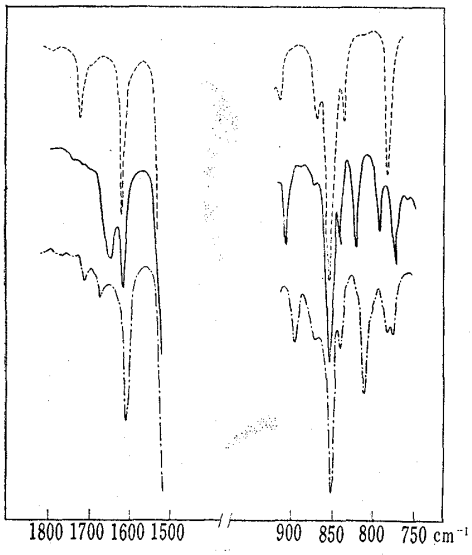


Fig. 3. Infrared Spectra (in Nujol)
(Nippon Bunko Model IR-S)

..... (XV) methiodide
——— (II) 〃
-·-·- (XVI) 〃

From the band at 820 and 813 cm^{-1} respectively in (II) and (XVI), each is assumed to have a trisubstituted ethylenic bond between 3 and 4 positions, and the absence of a band ascribable to the ethylenic bond in the infrared spectrum of (XV) appears not to contradict the presence of $-\overset{CH_3}{\overset{|}{C}}=C(OCH_3)-$ group at 2 and 3 positions. (II) and (XVI) probably represent stereoisomers due to different configuration of CH_3-group at 2-position.

Contrary to the three *syn*-dihydro-rotundines mentioned above, the natural dihydro-rotundine was found to be quite stable to 10% hydrochloric acid at elevated temperature. It was recovered quantitatively when heated with 10% hydrochloric acid on a steam-bath. Even after being refluxed with 10% hydrochloric acid in an oil-bath kept at 140°, the product recovered was proved to be the original substance, in which infrared inspection failed to trace a least amount of a ketonic substance. Thus, the presence of enol methyl ether group in natural dihydro-rotundine, as was reported by Matsuno, became highly improbable, and the structure of rotundine requires revision to be worked out by further detailed examination.

Experimental*3

2-Hydroxy-3-methyl-9,10-dimethoxy-6,7-dihydrobenzo[*a*]quinolizinium Chloride (IV) and its Corresponding Methyl-sulfonate——To a solution of 500 mg. of (III) in 18 cc. of 10% AcOH was added 1.187 g. of $(AcO)_2Hg$ and the whole was heated in a water bath kept at 90~95° for 3 hr., during which time scaly crystals of AcOHg precipitated out in a yellowish brown solution. The filtrate from AcOHg was heated and saturated with H_2S for 3 min. while hot to precipitate an excess Hg(II)

*3 A Perkin-Elmer Model 21 double-beam spectrophotometer was used for the determination of the infrared spectra and a Beckman Model DK-2 Spectrophotometer was used for the determination of the ultraviolet spectra.

as HgS, which was filtered together with added activated carbon. The filtrate was concentrated to 1/2 of the original volume and acidified with conc. HCl to pH 4.0～4.5. On ice-cooling, a crop of crystals came out, which was collected on a filter, washed with cold water, and recrystallized from 50% EtOH to 420 mg. (75%) of pure (IV) as colorless needles, m.p. 267～268°(decomp.), positive to $FeCl_3$ color reaction. *Anal.* Calcd. for $C_{16}H_{18}O_3NCl$: C, 62.43; H, 5.89; N, 4.55; Cl, 11.52. Found: C, 62.08; H, 6.12; N, 4.13; Cl, 11.52. UV λ_{max}^{EtOH} mμ (log ε): 218 (4.28), 237 (4.30), 257.5 (4.29), 318 (4.12). IR ν_{max}^{Nujol} cm^{-1}: 3280 (OH), 1630 (C=N).

A mixture of 100 mg. of (IV) and 1 cc. of Me_2SO_4 was heated in an oil bath kept at 140～145° for 30 min. Addition of Et_2O to the cooled reaction mixture gave 111 mg. of 2-hydroxy-3-methyl-9,10-dimethoxy-6,7-dihydrobenzo[*a*]quinolizinium methylsulfonate as a crystalline solid which, on recrystallization from MeOH, formed colorless needles, m.p. 264～265°(decomp.). *Anal.* Calcd. for $C_{14}H_{12}O_4NS(OMe)_3$: C, 53.25; H, 5.52; S, 8.36; OMe, 24.28. Found: C, 53.54; H, 5.69; S, 8.47; OMe, 23.32.

By adding excess of an aqueous solution of $NaHCO_3$ to a solution of (IV) in hot H_2O, crop of crystals came out, which, on recrystallization from dil. MeOH, formed colorless fine needles, m.p. 252～253.5°(decomp.). This seemed to be 2-hydroxy-3-methyl-9,10-dimethoxy-6,7-dihydrobenzo[*a*]-quinolizinium betain. UV λ_{max}^{EtOH} mμ (log ε): 218 (4.35), 237 (4.45), 262 (4.47), 310 (4.34). IR ν_{max}^{Nujol}: 1655 cm^{-1}(C=N), This betain reverted to the starting (IV) on treating with HCl.

3-Methyl-2,9,10-trimethoxy-6,7-dihydrobenzo[*a*]quinolizinium Chloride (VI)——A solution of 290 mg. of *rac*-dihydro-isorotundine (I) dissolved in 6 cc. of MeOH, and added with 654 mg. of $(AcO)_2Hg$ was warmed gently in a water bath with continuous stirring for 3 hr. The reaction mixture was filtered from precipitated AcOHg and a small amount of Hg, saturated with H_2S, and added with activated carbon. The precipitated HgS and carbon were filtered off, the filtrate was concentrated to 1/2 the original volume, saturated with dry HCl gas, and added with AcOEt. On cooling, (VI) crystallized out, which was collected, dried as usual, and recrystallized from MeOH-Et_2O mixture to 168 mg. of crystals, m.p. 249～251°(decomp.). On further recrystallization, this product formed, pale yellowish prisms, m.p. 252～253°(decomp.). *Anal.* Calcd. for $C_{14}H_{11}NCl(OMe)_3 \cdot \frac{1}{2}H_2O$; C, 61.72; H, 6.39; N, 4.23; Cl, 10.71; OMe, 28.14. Found: C, 61.69; H, 6.19; N, 4.42; Cl, 10.48; OMe, 28.25. UV λ_{max}^{EtOH} mμ (log ε): 219 (4.40), 236.5 (4.20), 257 (4.01), 345 (4.19). IR ν_{max}^{Nujol} 1626 cm^{-1}(C=N).

Reduction of 3-Methyl-2,9,10-trimethoxy-6,7-dihydrobenzo[*a*]quinolizinium Chloride (VI) with $NaBH_4$ in MeOH—— To a solution of 300 mg. of (VI) in 10 cc. of MeOH was added gradually 170 mg. of $NaBH_4$ at room temperature. The mixture was allowed to stand for 2 hr. and then warmed gently in water bath (40～45°) for 1.5 hr. with stirring. The reaction mixture was concentrated, added with a small amount of 2% NaOH solution, and extracted with Et_2O. The extract was dried over K_2CO_3 and the solvent was evaporated. The residue was distilled to give *rac*-dihydro-isorotundine (I) (108 mg.), b.$p_{0.001}$ 170°, which was characterized as its methiodide.
Methiodide: Colorless prisms (from MeOH-Et_2O), m.p. 226～227°(decomp.). IR ν_{max}^{Nujol}: 1672 cm^{-1} (C=C). *Anal.* Calcd. for $C_{15}H_{17}NI(OMe)_3$: C, 50.12; H, 6.07; N, 3.24; OMe, 21.58. Found: C, 49.66; H, 5.96; N, 3.02; OMe, 21.04.

2-Methyl-3-hydroxy-9,10-dimethoxy-6,7-dihydrobenzo[*a*]quinolizinium Chloride (X) and its Corresponding Betain——Dehydrogenation of 1.250 g. of 2-methyl-3-oxo compound (IX) dissolved in 20 cc. of 10% AcOH was carried out with 2.965 g. of $(AcO)_2Hg$ as in the case of dehydrogenation of (III) and 0.985 g. of the crude (X) was obtained, which, on recrystallization from 80% EtOH, formed pale yellowish prisms, m.p. 256～257°(decomp.), positive to $FeCl_3$ color reaction in H_2O. *Anal.* Calcd. for $C_{14}H_{12}ONCl(OMe)_2$: C, 62.43; H, 5.89; N, 4.55; Cl, 11.52; OMe, 20.16. Found: C, 62.20; H, 5.83; N, 4.62; Cl, 11.50; OMe, 20.02. UV λ_{max}^{EtOH} mμ (log ε): 245 (4.03), 257 (4.03), 280 (4.17), 318 (3.99), 366 (4.11). IR ν_{max}^{Nujol} cm^{-1}: 3280 (OH), 1635 (C=N).

A solution of 400 mg. of (X) in 4 cc. of H_2O was weakly basified with $NaHCO_3$ and extracted with $CHCl_3$, which was dried and the solvent was removed. The crude product formed 298 mg. of colorless prisms, m.p. 164～165°(decomp.), from EtOH-Et_2O mixture. UV λ_{max}^{EtOH} mμ (log ε): 245 (4.32), 246 (4.14), 279 (4.23), 322 (4.17). IR ν_{max}^{Nujol}: 1657 cm^{-1}(C=N).

This product was considered to be 2-methyl-3-hydroxy-9,10-dimethoxy-6,7-dihydrobenzo[*a*]quinolizinium betain, because (X) was recovered on being treated with HCl.

2-Methyl-3,9,10-trimethoxy-6,7-dihydrobenzo[*a*]quinolizinium Chloride (XII) (Synthetic Dehydrorotundinium Chloride)——A solution of 670 mg. of *rac*-dihydro-rotundine (II) in 15 cc. of MeOH was added with 1.505 g. of $(AcO)_2Hg$ and the mixture was heated in a water bath kept at 65° for 2.5 hr. with stirring, during which time AcOHg and a small amount of Hg precipitated out and the solution turned deep yellowish brown. The filtrate from the precipitate was saturated with H_2S to remove excess Hg(II) as HgS, which was filtered off with the aid of added activated carbon. The clear filtrate was concentrated, added with a small amount of Me_2CO, and strongly acidified with conc. HCl under ice-cooling. On keeping the acidified solution in a refrigator, 411 mg. of crude (XII) came out as pale yellowish green crystals, which were collected, washed with Me_2CO, and dried. The crude (XII), m.p. 227～231°(decomp.), thus obtained was recrystallized from MeOH-iso-Pr_2O mixture

to pale yellowish green prisms, m.p. 234~235°(decomp.), negative to $FeCl_3$ color reaction. *Anal.* Calcd. for $C_{14}H_{11}NCl(OMe)_3 \cdot H_2O$: C, 60.08; H, 6.22; N, 4.11; Cl, 10.42; OMe, 27.39. Found : C, 59.71; H, 6.60; N, 4.02; Cl, 10.20; OMe, 25.95. UV λ_{max}^{EtOH} mμ (log ε) : 271 (4.20), 286 (4.21), 362 (4.14). IR ν_{max}^{Nujol} : 1632 cm^{-1}(C=N).

Methylation of (X) with Dimethyl Sulfate; Preparation of Synthetic Dehydro-rotundinium Methylsulfonate (XIV)——To a suspension of 600 mg. of (X) in 1.5 cc. of 33% dil. KOH solution was added 0.9 cc. of Me_2SO_4 at room temperature (25°) with continuous agitation and the mixture began to react with evolution of heat. The reaction temperature was controlled at 20~40° with ice-cooling. Five min. later, Me_2SO_4 added was consumed. To ensure complete methylation, this procedure was repeated twice, using 1.5 cc. of 33% KOH and 0.9 cc. of Me_2SO_4 each time. Finally, 4.5 cc. of 33% KOH was added to decompose excess Me_2SO_4, during which time crystals began to separate. After the whole mixture was allowed to stand at room temperature with continuous stirring for additional 2.5 hr., the crystals were collected by filtration and dissolved in $CHCl_3$. The $CHCl_3$ solution was dried over dehyd. Na_2SO_4, acidified with methanolic HCl, and concentrated to give yellow crystals, which were recrystallized from MeOH-iso-Pr_2O mixture to 612 mg. of brilliant yellowish green needles, m.p. 245~246° (decomp.). *Anal.* Calcd. for $C_{14}H_{11}O_3NS(OMe)_4$: C, 54.39; H, 5.83; N, 3.52; S, 8.06; OMe, 31.23. Found : C, 53.98; H, 6.06; N, 3.75; S, 8.11; OMe, 29.55.

The methylsulfonate thus obtained was converted to the corresponding iodide (XIII) by the usual process, and recrystallized from 50% MeOH to form pale brownish yellow needles, m.p. 267~268° (decomp.). *Anal.* Calcd. for $C_{14}H_{11}NI(OMe)_3$: C, 49.40; H, 4.87; N, 3.38; I, 30.71; OMe, 22.52. Found: C, 49.39; H, 4.96; N, 3.68; I, 30.41; OMe, 21.83. UV λ_{max}^{EtOH} mμ (log ε) : 272 (4.20), 286 (4.22), 365 (4.14). IR ν_{max}^{Nujol} : 1635 cm^{-1}(C=N).

The iodide (XIII) formed was converted to the corresponding chloride of m.p. 235~236°(decomp.) and was identified with synthetic dehydro-rotundinium chloride (XII).

Dehydrogenation of Dihydro-rotundine Base, the Reduction Product of *l*-Rotundine, with $(AcO)_2$-Hg; Preparation of Dehydro-rotundinium Chloride——To a solution of 20 mg. of dihydro-rotundine in 0.5 cc. of MeOH, was added 45.14 mg. of $(AcO)_2Hg$ and the whole mixture was allowed to stand at room temperature (25°) for 10 min., followed by heating in a water bath kept at 70° for 3 hr. with continuous stirring, during which time AcOHg began to precipitate out and the solution turned yellowish brown. The reaction mixture was filtered and the filtrate was saturated with H_2S. After adding activated carbon to the mixture, the mixture was filtered to give a clear filtrate. On adding methanolic HCl to the filtrate, 15.03 mg. of crystals precipitated out, which were collected, dried, and recrystallized from MeOH-iso-Pr_2O mixture to 12.16 mg. of brownish yellow crystals, m.p. 203~204°(decomp.). On further recrystallization, melting point was raised to 205~206°. UV λ_{max}^{EtOH} mμ (log ε) : 228 (4.28), 266 (4.29), 274 (4.29), 347 (4.31), log ε being calculated by estimating the product to have the same molecular formula as synthetic dehydro-rotundinium chloride.

Reduction of Synthetic Dehydro-rotundinium Salt with $NaBH_4$; Preparation of the Base (XV)——To a solution of 200 mg. of methylsulfonate (XIV) in 10 cc. of MeOH was added gradually 152 mg. of $NaBH_4$ at room temperature. After being allowed to stand for 2 hr., the whole was gently refluxed to give an almost colorless solution. The reaction mixture was concentrated, added with small amount of H_2O, and extracted with iso-Pr_2O. The extract was concentrated, after being dried over dehydrated Na_2SO_4 and K_2CO_3. The residue was extracted with hexane, the extract was purified through Al_2O_3 chromatography, and the effluent was concentrated to give 83 mg. of a syrupy residue which soon solidified. The solid product thus obtained was recrystallized from hexane to pure (XV) as colorless needles, m.p. 98~99°. *Anal.* Calcd. for $C_{17}H_{23}O_3N$: C, 70.55; H, 8.01. Found : C, 70.74; H, 8.63. UV λ_{max}^{EtOH} : 283 mμ (log ε 3.57).

On similar treatment of 200 mg. of the chloride (XII) in 10 cc. of MeOH, using 143 mg. of $NaBH_4$ as a reducing agent, 121.5 mg. of the same base (XV) was obtained. The methiodide of (XV) was prepared by the usual method and recrystallized from MeOH to colorless prisms, m.p. 248~249°. *Anal.* Calcd. for $C_{18}H_{26}O_3NI$: C, 50.12; H, 6.07; N, 3.24; I, 29.42. Found : C, 50.28; H, 6.42; N, 3.18; I, 29.12. UV λ_{max}^{EtOH} : 283 mμ (log ε 3.62).

Dehydrogenation of (XV) with $(AcO)_2Hg$——Fifty milligrams of (XV) dissolved in 1.25 cc. of MeOH was dehydrated with 112.85 mg. of $(AcO)_2Hg$ at room temperature followed by boiling in a water bath kept at 60~62° for 1 hr. with continuous stirring. The whole reaction mixture was filtered from the precipitated AcOHg and the filtrate was saturated with H_2S. The precipitated HgS was filtered off with the aid of activated carbon. The filtrate was concentrated, acidified with conc. HCl (crystalline precipitation appeared), added with $CHCl_3$ and MeOH, and concentrated further to dryness. The residual crystals were recrystallized from MeOH-iso-Pr_2O mixture to 23 mg. of the chloride as deep greenish yellow crystals, m.p. 229~231°(decomp.), which were converted to the iodide by the usual procedure. The iodide, m.p. 266~267.5°(decomp.), was identified with the above-described synthetic dehydro-rotundinium iodide (XIII) by its physicochemical properties.

Hydrolysis of (XV)——A solution of 20 mg. of the base (XV) in 1 cc. of 10% HCl was heated in a water bath for 1 hr., followed by concentration *in vacuo*. The residue was dissolved in a small amount of H_2O, basified with K_2CO_3, and extracted with benzene. The extract was washed with 10% NaOH and H_2O, dried over dehyd. Na_2SO_4, and concentrated to give 13.2 mg. of a pale yellowish oil (IX), which was converted to the methiodide by the usual procedure. The methiodide thus obtained was recrystallized from MeOH to pale yellow prisms, m.p. 232~233.5° (decomp.). *Anal.* Calcd. for $C_{17}H_{24}O_3NI \cdot \frac{1}{2}H_2O$: C, 47.89; H, 5.91; N, 3.28. Found: C, 47.56; H, 5.72; N, 3.22. IR ν_{max}^{Nujol}: 1725 cm^{-1} (C=O).

Dehydration of 108 mg. of the oxo compound (IX) thus obtained in 2 cc. of 10% AcOH was carried out as described above, using 256 mg. of $(AcO)_2Hg$. The reaction mixture was treated with H_2S as before and the filtrate was acidified with conc. HCl to give 68.3 mg. of pale yellow prisms, m.p. 252~253° (decomp.), positive to $FeCl_3$ color reaction. Melting point of this product was raised to 254~255° (decomp.) through recrystallization from 80% EtOH and the product was identified with the above-mentioned (X). IR ν_{max}^{Nujol} cm^{-1}: 3270 (OH), 1635 (C=N).

Catalytic Reduction of Quaternary Ammonium Base (XII)——A solution of 200 mg. of (XII) in 10 cc. of MeOH, added with 50 mg. of NaOH dissolved in 0.5 cc. of H_2O, was reduced over 60 mg. of PtO_2 catalyst as atmospheric pressure. Reduction was almost completed after 27.9 cc. of H_2 had been absorbed (about 40 min.) and the solution turned colorless from deep greenish yellow. The solution was filtered, and MeOH was evaporated *in vacuo*. The residue was added with cold H_2O and extracted with iso-Pr_2O. The extract, after being dried over anhyd. K_2CO_3, was concentrated *in vacuo*, the residue was dissolved in hexane, and purified through Al_2O_3 chromatography. From the effluent, 109 mg. of pale yellowish syrupy oil (XVI) was obtained, which was converted to the methiodide as pale yellowish prisms (from MeOH), m.p. 232~233° (decomp.). *Anal.* Calcd. for $C_{18}H_{26}O_3NI \cdot \frac{1}{2}H_2O$: C, 49.09; H, 6.18; N, 3.18; I, 28.82. Found: C, 48.80; H, 6.03; N, 3.25; I, 28.64. IR ν_{max}^{Nujol}: 1665 cm^{-1} (C=C).

Hydrolysis of the Base (XVI)——A solution of 148 mg. of the base (XVI), prepared as above, dissolved in 7 cc. of 10% HCl was heated in a boiling water bath for 1.5 hr. The reaction mixture was worked up as usual and the product (IX) was converted to the methiodide, which recrystallized from MeOH as pale yellowish prisms, m.p. 232~233° (decomp.). *Anal.* Calcd. for $C_{17}H_{24}O_3NI \cdot \frac{1}{2}H_2O$: C, 47.89; H, 5.91; N, 3.28. Found: C, 47.94; H, 5.91; N, 3.24. IR ν_{max}^{Nujol}: 1725 cm^{-1} (C=O).

Hydrolysis of *rac*-Dihydro-rotundine (II)——(II) (198 mg.) was hydrolyzed with 15 cc. of 10% HCl. The reaction mixture was treated as usual and 153 mg. of brownish oil (IX) was obtained. This was converted to the methiodide, as pale yellowish prisms (from MeOH), m.p. 232~233° (decomp.). *Anal.* Calcd. for $C_{17}H_{24}O_3NI \cdot \frac{1}{2}H_2O$: C, 47.89; H, 5.91; N, 3.28. Found: C, 47.66; H, 6.15; N, 3.14. IR ν_{max}^{Nujol}: 1672 cm^{-1} (C=O).

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Summary

The proposed structure for rotundine and dihydro-rotundine by Matsuno was proved untenable. The synthesized so-called *rac*-dihydro-rotundine (II) was dehydrogenated with mercuric acetate in methanolic solution to give the product as well-defined quaternary salt (XII). This was more advantageously prepared by dehydrogenating 2-oxo-3-methyl-9, 10-dimethoxy-1, 2, 3, 4, 6, 7-hexahydro-11b*H*-benzo[*a*]quinolizine (IX), the intermediate for (II), with mercuric acetate in 10% acetic acid solution to produce a phenolic quaternary salt (X) and methylating the latter with dimethyl sulfate and an excess of conc. potassium hydroxide solution, giving (XIV). Natural dihydro-rotundine, prepared by reducing the natural alkaloid, gave a dehydro-quaternary salt by a similar method, but the product showed ultraviolet and infrared absorption spectra different from those of (XII). Partial hydrogenation experiments of (XII) and (XIV) under a variety of conditions failed to afford a compound having the diene system, as was proposed for natural rotundine. The products isolated were *rac*-dihydro-rotundine-type of compound, whose three

isomers (II, XV, and XVI) were characterized. In conformity with their structure they smoothly gave the ketone (IX) when treated with dil. hydrochloric acid, whereas the natural dihydro-rotundine was proved to be quite stable to hydrochloric acid even at an elevated temperature.

Similar experiments were also conducted with synthetic *rac*-dihydro-isorotundine (I).

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33. Masuo Akagi and Isamu Aoki : Studies on Food Additives. VIII.[1] Metabolism of α-Hydroxy-2,6-di-*tert*-butyl-*p*-cresol. Isolation of Metabolites.

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In the previous work[1] of this series, a glucuronide as its methyl acetate derivative was isolated from the urine of a rabbit receiving 2,6-di-*tert*-butyl-*p*-cresol (BHT) and Aoki assumed its structure as methyl [(3,5-di-*tert*-butyl-4-hydroxybenzyl)-2,3,4-tri-O-acetyl-β-D-glucopyranosid]uronate.

In the present series of work, as a means for certifying the structure of its glucuronide, the isolation of metabolites from the urine of a rabbit receiving α-hydroxy-2,6-di-*tert*-butyl-*p*-cresol (BHT-alc) was carried out, and unchanged BHT-alc (M_1), 3,5-di-*tert*-butyl-4-hydroxybenzaldehyde (BHT-ald, M_2) as its 2,4-dinitrophenylhydrazone, 3,5-di-*tert*-butyl-4-hydroxy-benzoic acid (BHT-acid, M_3), 4,4′-ethylenebis(2,6-di-*tert*-butylphenol) (BHT-diphenylethane, M_4), were isolated, similarly as in the case[1] of BHT-dosed rabbits, and also a glucuronide (M_5) as its methyl acetate derivative.

This glucuronide was clearly different from the glucuronide[1] isolated from BHT-dosed rabbits or synthesized methyl [(3,5-di-*tert*-butyl-4-hydroxy-benzoyl)-2,3,4-tri-O-acetyl-β-D-glucopyranosid]uronate.

Experimental

Materials——BHT-ald was prepared by oxidation of BHT in a mixture of AcOH-H_2O (5:1) with Br_2 according to the method of Fujisaki.[2]

BHT-alc was prepared as follows : BHT-ald (3 g.) was suspended in 30 cc. of MeOH with stirring and $NaBH_4$ (0.5 g. in 5 cc. of 0.1*N* NaOH) was added to this suspension in small portions at room temperature. After standing overnight at room temperature, the white crystals that separated were collected and washed with a small amount of H_2O. Recrystallization from 100 cc. of ligroine (b.p. 80~120°) gave colorless crystals (2.8 g.), m.p. 137~138° (reported[*2] m.p. 137°). *Anal.* Calcd. for $C_{15}H_{24}O_2$: C, 76.22; H, 10.24. Found : C, 76.34; H, 10.17.

The preparation of BHT-acid and BHT-diphenylethane was described in the previous paper.[3]

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*2 This compound was prepared through reduction of BHT-ald with $LiAlH_4$ by Coppinger. *et al.* [J. Am. Chem. Soc., **75**, 734 (1953)] and through hydrolysis of α-bromo-2,6-di-*tert*-butyl-*p*-cresol in H_2O-Me_2CO by Cook, *et al.* (*Ibid.*, **77**, 1783 (1955)).

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