acetate (2.50 g, 6.4 mmoles) was mixed with 5.0 g of crystalline phosphoric acid[17] which had been dried overnight *in vacuo* over magnesium perchlorate. The mixture was stirred magnetically *in vacuo* (oil pump) at 85° for 0.75 hr, and the resulting very dark brown syrup was cooled and to it was added 100 ml of ice-cold 2 *N* lithium hydroxide. The contents of the flask were mixed by vigorous shaking, and the flask was then heated on the steam bath for 2.5 hr to hydrolyze any polyphosphates. Precipitated lithium phosphate was removed from the cooled mixture by filtration through Celite and washed with *ca.* 0.01 *N* lithium hydroxide; the dark brown filtrate contained 2.87 mmoles of phosphate (45%). Dowex 50W-H$^+$ was used to adjust the pH to about 9 and, after the resin had been removed by filtration, 1.5 g of barium acetate was added. The solution was concentrated *in vacuo* to 50 ml, a trace of precipitate was removed by centrifugation, and the barium salt was precipitated with three volumes of ethanol. After several hours at 5°, the salt was collected by centrifugation and washed with 75% ethanol, acetone, and ether and dried over calcium chloride *in vacuo*. This precipitation was repeated four times, twice using 50 ml of water and twice using 100 ml of water; only in the final precipitation was there no water-insoluble barium salt to be removed by centrifugation. The barium salt (0.98 g) was dissolved in water, the solution was passed through a cooled column of Dowex 50W-H$^+$ (1 × 25 cm) into water containing 0.5 g of potassium hydroxide, and the column was washed with 75 ml of water. The pH of the resulting solution was adjusted to 9 with Dowex 50W-H$^+$ and the resin was filtered off; the solution was then treated with charcoal and concentrated at reduced pressure to 20 ml. The potassium salt which crystallized at 5° by the gradual addition of 2.5 volumes of ethanol over a period of several days was collected by filtration and dried *in vacuo* over calcium chloride at 15 mm. A second crystallization performed in the same manner gave 0.51 g (21%) of almost pure dipotassium α-D-galactopyranose 1-phosphate dihydrate, $[\alpha]^{26}D$ +96°, lit.[22] $[\alpha]^{26}D$ +98°.

(22) H. W. Kosterlitz, *Biochem. J.*, **33**, 1087 (1939).

The Structure and Total Synthesis of Takatonine[1]

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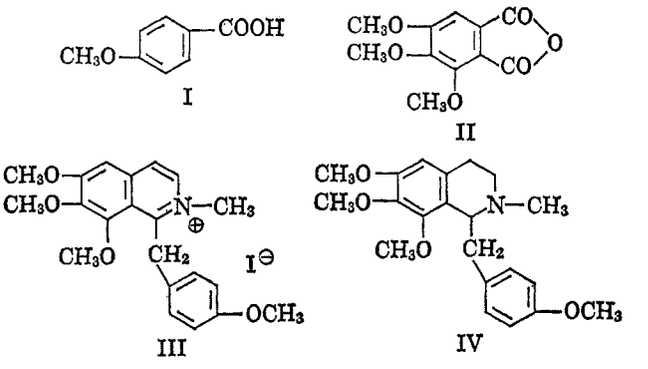
Total syntheses of takatonine iodide and tetrahydrotakatonine by unequivocal routes show that takatonine iodide should be assigned the 1-(4′-methoxybenzyl)-5,6,7-trimethoxyisoquinoline methiodide structure (VI), rather than the isomeric 6,7,8-trimethoxy structure III considered earlier.

Takatonine is a quarternary base isolated from the Japanese commercial crude drug "Takato-gusa," the dried leaves and stems of *Thalictrum minus*. It is the purpose of this paper to present, in detail, the elucidation of structure VI and the total synthesis of takatonine iodide. Takatonine appears to be the first benzylisoquinoline alkaloid recognized to contain a substituent at C-5.[3]

In an earlier study,[4] Hofmann degradation of tetrahydrotakatonine and permanganate oxidation of the resulting methine base afforded anisic acid (I) and an amino acid. The second Hofmann degradation of the amino acid followed by permanganate oxidation afforded 3,4,5-trimethoxyphthalic anhydride (II). On the basis of the latter results and some spectral data, the structure, 1-(4′-methoxybenzyl)-2-methyl-6,7,8-trimethoxyisoquinoline iodide (III), was tentatively assigned to takatonine iodide. However, the location of the methoxyl group at position 8 of isoquinoline seemed equivocal, because of the absence of firm chemical evidence (see Chart I).

In the course of an investigation of the structure of cissampareine,[5] several substituted benzyltetrahydroisoquinoline derivatives were synthesized for comparison with derivatives of the sodium–liquid ammonia cleavage products. It was found that the thin layer chromatographic behavior and infrared and n.m.r. spectra of tetrahydrotakatonine were not identical with those of compound IV which was synthesized by the method of Tomita and Okui.[6] The n.m.r. spectrum of tetrahydrotakatonine (see Figure 1) showed four methoxyl signals at τ 6.15 (6 H), 6.22 (3 H), and 6.43 (3 H), and one N-methyl signal at 7.48 (3 H), while that of synthetic compound IV (see Figure 2) showed four methoxyl signals at τ 6.05, 6.15, 6.18, and 6.23, and one N-methyl signal at 7.65. Moreover, in the spectrum of the former, a singlet proton signal at τ 4.12 was observed, while in that of the latter a singlet at 3.63 was found. Analysis[7] of these n.m.r. spectra, and the chemical results described above, led

CHART I



(1) This is part IV of a series entitled *Thalictrum* Alkaloids; part III, S. M. Kupchan and N. Yokoyama, *J. Am. Chem. Soc.*, **86**, 2177 (1964). This is also part XVII of a series entitled Studies on the Alkaloids of *Thalictrum thunbergii* DC.; part XVI, E. Fujita, K. Fuji, and T. Suzuki, *Bull. Inst. Chem. Res. Kyoto Univ.*, in press. For a preliminary publication, see S. Kubota, T. Masui, E. Fujita, and S. M. Kupchan, *Tetrahedron Letters*, 3599 (1965).

(2) Faculty of Pharmacy, University of Tokushima, Sho-machi, Tokushima, Japan.

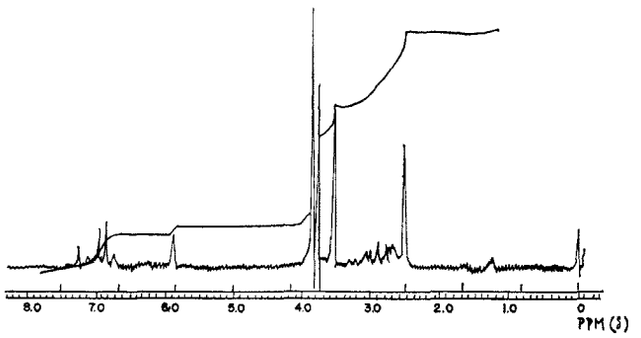
(3) The phenolic benzyltetrahydroisoquinoline alkaloid, thallifendlerine, recently has been shown to possess the same substitution pattern, by methylation to an O-methyl derivative which corresponds to optically active tetrahydrotakatonine: M. Shamma, M. A. Greenberg, and B. S. Dudock, *Tetrahedron Letters*, 3595 (1965).

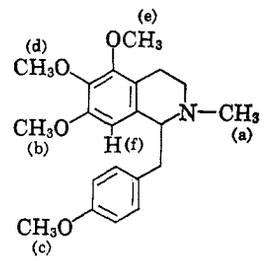
(4) E. Fujita and T. Tomimatsu, *Yakugaku Zasshi*, **79**, 1082 (1959).

(5) S. M. Kupchan, A. C. Patel, and E. Fujita, *J. Pharm. Sci.*, **54**, 580 (1965); S. M. Kupchan, S. Kubota, E. Fujita, S. Kobayashi, J. H. Block, and S. A. Telang, in press.

(6) M. Tomita and K. Okui, *Yakugaku Zasshi*, **76**, 632 (1956).

(7) M. Tomita, T. Shingu, K. Fujitani, and H. Furukawa, *Chem. Pharm. Bull.* (Tokyo), **13**, 921 (1965).





V

Assignments, τ values

a, 7.48	d, 6.15
b, 6.43	e, 6.15
c, 6.22	f, 4.12

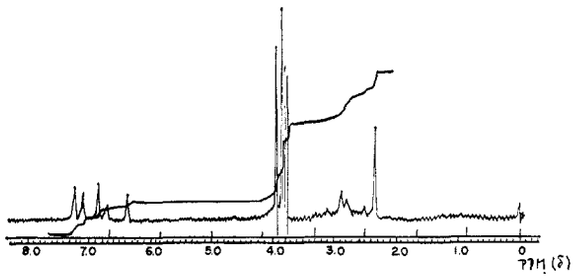
Figure 1.—Nuclear magnetic resonance spectrum of tetrahydrotakatonine (V).

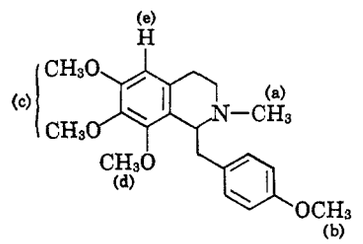
to the postulation that takatonine must have three methoxyl groups at positions 5, 6, and 7, rather than 6, 7, and 8 of the isoquinoline moiety.

For comparison with tetrahydrotakatonine and takatonine iodide, 1-(4′-methoxybenzyl)-2-methyl-5,-6,7-trimethoxy-1,2,3,4-tetrahydroisoquinoline (V) and 1 - (4′ - methoxybenzyl) - 5,6,7 - trimethoxyisoquinoline methiodide (VI) were synthesized by the following routes. Lithium aluminum hydride reduction of β-nitrostyrene (VIII), obtained by condensation of 2,3,4-trimethoxybenzaldehyde (VII)[8] with nitromethane, gave 2,3,4-trimethoxyphenethylamine (IX). Schotten–Baumann reaction of IX with 4-methoxyphenylacetyl chloride yielded phenylacetamide X. Bischler–Napieralski cyclization of compound X afforded 1-(4′-methoxybenzyl)-5,6,7-trimethoxy-3,4-dihydroisoquinoline (XI). The latter was found to be oxidized easily to the ketone XIII, upon treatment with 10% sodium hydroxide solution, or attempted purification by column chromatography on alumina, as in the cases of 1-(2′,3′-dimethoxybenzyl)-5,6-dimethoxyisoquinoline,[9] 1-(3′-methoxybenzyl)-6-methoxy-3,4-dihydroisoquinoline,[10] and 3,4-dihydropapaverine.[11] The isolation of the free base XI from the acidic cyclization reaction mixture, was achieved by a rapid treatment with ammonia under nitrogen and ether extraction. Sodium borohydride reduction of the ketone XIII gave 1-(4′-methoxy-α-hydroxybenzyl)-5,6,7-trimethoxy-1,2,3,4-tetrahydroisoquinoline (XIV), whose N-methylated derivative XV was also prepared.

The dihydroisoquinoline XI on reduction with sodium borohydride yielded 1-(4′-methoxybenzyl)-5,6,7-trimethoxy-1,2,3,4-tetrahydroisoquinoline (XII). N-Methylation with formalin and sodium borohydride gave 1-(4′-methoxybenzyl)-5,6,7-trimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline (V). Sodium borohydride reduction of methiodide XVI derived from compound XI also yielded compound V. Comparison of infrared and n.m.r. spectra (see Figure 1) and R_f values upon thin layer chromatography on alumina showed tetrahydrotakatonine to be identical with synthetic tetrahydroisoquinoline V. The identity was also confirmed by infrared spectral and mixture melting point determinations of their hydrochlorides and picrates.

The tetrahydroisoquinoline XII was dehydrogenated to 1-(4′-methoxybenzyl)-5,6,7-trimethoxyisoquinoline



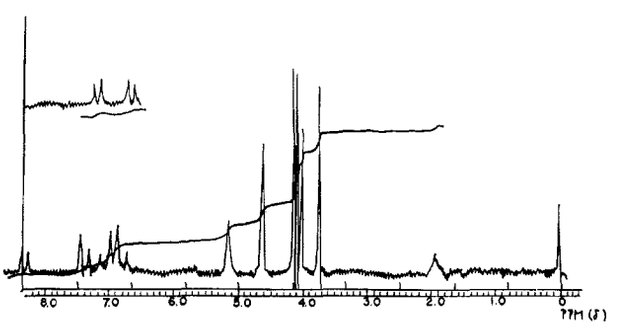


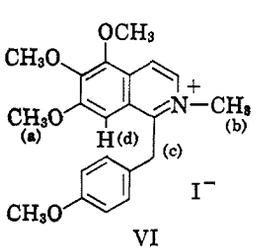
IV

Assignments, τ values

a, 7.65	d, 6.05
b, 6.23	e, 3.63
c, 6.15, 6.18	

Figure 2.—Nuclear magnetic resonance spectrum of synthetic compound IV.





VI

Assignments, τ values

a, 6.24	c, 4.86
b, 5.38	d, 2.56

Figure 3.—Nuclear magnetic resonance spectrum of takatonine iodide (VI).

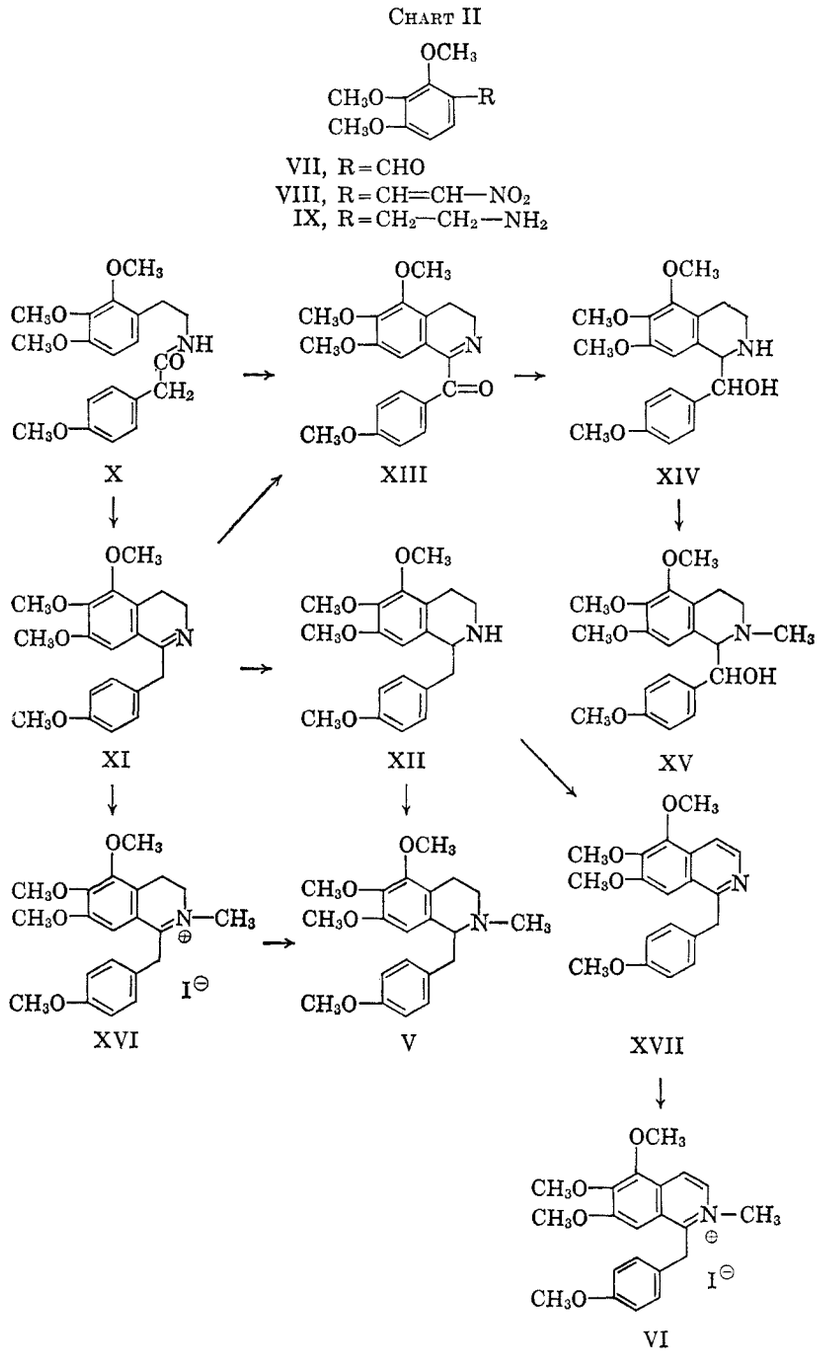
(8) P. E. Papadakis and W. Boand, *J. Org. Chem.*, **26**, 2075 (1961).

(9) E. Späth, K. Riedel, and G. Kubiczek, *Monatsh.*, **79**, 72 (1948).

(10) R. S. Livshits, G. I. Bazilevskaya, M. S. Bainova, O. E. Dobrovinskaya, and N. A. Preobrazhenskii, *Zh. Obshch. Khim.*, **17**, 1671 (1947); *Chem. Abstr.*, **42**, 2606 (1948).

(11) T. Kametani and K. Fukumoto, *Yakugaku Zasshi*, **83**, 1031 (1963).

CHART II



(XVII) by refluxing in decalin with palladium black at 180–240° under nitrogen. The isoquinoline XVII was refluxed with methyl iodide in methanol under nitrogen to give methiodide VI in the form of yellow crystals. The product was shown to be takatonine iodide by mixture melting point and infrared, ultraviolet and n.m.r. (see Figure 3) spectral comparison with the alkaloid (see Chart II).

Thus, the total synthesis of takatonine by an unequivocal route was accomplished and the structures of takatonine iodide and tetrahydrotakatonine were shown to be VI and V, respectively.

Experimental Section[12]

2,3,4-Trimethoxy-β-nitrostyrene (VIII).—A mixture of 2,3,4-trimethoxybenzaldehyde (VII, 5 g.), nitromethane (3 ml.), glacial acetic acid (10 ml.), and ammonium acetate (1.6 g.) was refluxed for 2 hr. The crude yellow product was recrystallized from methanol to give 3.7 g. of yellow needles, m.p. 77–78°. *Anal.* Calcd. for $C_{11}H_{13}NO_5$: C, 55.00; H, 5.40; N, 5.86. Found: C, 55.18; H, 5.47; N, 6.09.

2,3,4-Trimethoxyphenethylamine (IX).—The reduction of the β-nitrostyrene VIII with lithium aluminum hydride was carried out by general method described by Ramirez and Burger.[13] To a well stirred suspension of 15.5 g. of lithium aluminum hydride in 100 ml. of dry ether was added dropwise a solution of 18.8 g. of nitrostyrene VIII in 300 ml. of dry ether, and the mixture was refluxed for 2 hr. Excess lithium aluminum hydride was decomposed by adding ice-cold 10% sulfuric acid dropwise with cooling. The water layer was separated and its pH was adjusted to 6 with solid lithium carbonate. The solution was heated to boiling, and the aluminum hydroxide which precipitated was centrifuged and separated. The clear solution, combined with washings, was mixed with a solution of picric acid in the minimum amount of hot ethanol. Upon standing overnight at room temperature, yellow picrate was obtained.

(12) All melting points are uncorrected.

(13) F. A. Ramirez and A. Burger, *J. Am. Chem. Soc.*, **72**, 2781 (1950).

Recrystallization from methanol gave crystals of m.p. 139–140°. *Anal.* Calcd. for $C_{17}H_{20}N_4O_{10}$: C, 46.36; H, 4.58; N, 12.72. Found: C, 46.49; H, 4.80; N, 12.19.

N-(2,3,4-Trimethoxyphenethyl)-4′-methoxyphenylacetamide (X).—The phenethylamine IX was freed from the picrate (5 g.) with 5% sodium hydroxide solution, and extracted with chloroform. Washing with saturated sodium chloride solution and drying, followed by evaporation gave a residue which was dissolved in ether. A solution of 2.9 g. of 4-methoxyphenylacetyl chloride in 12 ml. of ether was added dropwise to a well-stirred mixture of ethereal solution of phenethylamine IX and 5% sodium hydroxide solution (50 ml.). After stirring at room temperature for 1 hr., the reaction mixture was extracted with chloroform. The extract was washed with water, dilute hydrochloric acid, and again water, then dried over anhydrous sodium sulfate. The solvent was distilled off to give crude, pale yellow crystals which were recrystallized from petroleum ether and ethyl acetate to yield colorless needles (3.2 g.), m.p. 77–78°, infrared spectrum $\nu_{max}^{CHCl_3}$ 1655 and 3420 cm.$^{-1}$. *Anal.* Calcd. for $C_{20}H_{25}NO_5$: C, 66.83; H, 7.01; N, 3.90. Found: C, 67.21; H, 7.20; N, 4.19.

1-(4′-Methoxybenzyl)-5,6,7-trimethoxy-3,4-dihydroisoquinoline (XI).—A mixture of 5 g. of the amide X in 40 ml. of dried toluene and phosphorus oxychloride (7.2 ml.) was heated on an oil bath at 100° for 1 hr. Toluene and excess phosphorus oxychloride were distilled off *in vacuo*. The brown residue was washed twice with petroleum ether, then dissolved in water (20 ml.). The solution under nitrogen was made alkaline with 5% ammonium hydroxide and rapidly extracted with ether. The ethereal solution was washed with water, dried over anhydrous sodium sulfate, and evaporated under nitrogen to give an oil (4.2 g.). The hydrochloride had m.p. 146.5–149.5° (recrystallized from acetone), ultraviolet spectrum λ_{max}^{MeOH} 272 mμ (log ε 3.96) and 315 mμ (log ε 3.43). The picrate had m.p. 170–172° (from isopropyl ether). *Anal.* Calcd. for $C_{26}H_{26}N_4O_{11}$: C, 54.73; H, 4.59; N, 9.82. Found: C, 54.74; H, 4.44; N, 9.70.

1-(4′-Methoxybenzyl)-5,6,7-trimethoxy-1,2,3,4-tetrahydroisoquinoline (XII).—To a solution of 1 g. of dihydroisoquinoline XI in 10 ml. of methanol was added 1 g. of sodium borohydride during 0.5 hr. under stirring. After additional stirring for 40 min., methanol was distilled off. The residue was treated with water and basified with ammonium hydroxide. The base was extracted with ether repeatedly and the extract was washed well with water. After drying over anhydrous sodium sulfate, the solution was evaporated to dryness. Recrystallization of the crystalline residue from acetone and petroleum ether (b.p. 35–45°) gave colorless crystals, m.p. 85–87°. The hydrochloride was recrystallized from isopropyl alcohol, m.p. 181–185°, ultraviolet spectrum λ_{max}^{MeOH} 278 mμ (log ε 3.54) and 284 mμ (log ε 3.51). *Anal.* Calcd. for $C_{20}H_{26}ClNO_4$: C, 63.24; H, 6.85; N, 3.68. Found: C, 63.08; H, 7.37; N, 3.32. The picrate had m.p. 180.5–182.5° (from methanol). *Anal.* Calcd. for $C_{26}H_{29}N_4O_{11}$: C, 54.55; H, 4.89; N, 9.79. Found: C, 54.72; H, 4.95; N, 9.68.

1-(4′-Methoxybenzoyl)-5,6,7-trimethoxy-3,4-dihydroisoquinoline (XIII).—A mixture of 1.9 g. of amide X in 16 ml. of dried toluene and 3 g. of phosphorus oxychloride was heated in an oil bath at 120–125° for 1 hr. After removing toluene and excess phosphorus oxychloride by distillation *in vacuo*, the brown oily residue was washed with petroleum ether, and dissolved in water. The solution was made alkaline with 10% sodium hydroxide solution and extracted with ether. The ethereal extract was dried over anhydrous sodium sulfate, and evaporated to dryness. Recrystallization from benzene gave colorless needles (0.82 g.), m.p. 143–145.5°, ultraviolet spectrum λ_{max}^{MeOH} 296.5 mμ (log ε 4.35), infrared spectrum $\nu_{max}^{CHCl_3}$ 1663 (C=O) and 1615 cm.$^{-1}$ (C=N) (shoulder). *Anal.* Calcd. for $C_{20}H_{21}NO_5$: C, 67.59; H, 5.91; N, 3.94. Found: C, 67.52; H, 5.85; N, 4.01. The picrate gave yellow needles (from methanol), m.p. 135–137°, infrared spectrum ν_{max}^{KBr} 1680 cm.$^{-1}$ (C=O). *Anal.* Calcd. for $C_{26}H_{24}N_4O_{12}$: C, 53.43; H, 4.14; N, 9.59. Found: C, 53.66; H, 4.37; N, 9.35.

1-(4′-Methoxy-α-hydroxybenzyl)-5,6,7-trimethoxy-1,2,3,4-tetrahydroisoquinoline (XIV).—Sodium borohydride (320 mg.) was added to the benzoyldihydroisoquinoline XIII (250 mg.) in methanol during 1 hr. with stirring at room temperature. After stirring for 1 additional hr., the solvent was distilled off to give a residue which was suspended in water and made alkaline with 2% sodium hydroxide solution. Extraction with ether followed by the usual treatment and crystallization from ether afforded colorless needles (180 mg.), m.p. 119.5–121.5°, ultraviolet spectrum λ_{max}^{MeOH} 276.5 mμ (log ε 3.52) and 282.5 mμ (log ε 3.51). *Anal.* Calcd. for $C_{20}H_{25}NO_5$: C, 66.83; H, 7.01; N, 3.90. Found: C, 66.57; H, 6.81; N, 4.14.

1-(4′-Methoxy-α-hydroxybenzyl)-5,6,7-trimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline (XV).—A solution of 150 mg. of tetrahydroisoquinoline XIV in 6 ml. of methanol was stirred with 1 ml. of 37% formalin at room temperature for 0.5 hr. Sodium borohydride (180 mg.) was added, and the solution was stirred for an additional 0.5 hr. After the solvent was removed by distillation, the residue was basified with 2% sodium hydroxide solution, extracted with ether, and washed with water. The ethereal extract was dried over anhydrous potassium carbonate and evaporated to dryness. Recrystallization from ligroin gave colorless crystals (60 mg.), m.p. 101–102°, infrared spectrum $\nu_{max}^{CHCl_3}$ 3420 cm.$^{-1}$. *Anal.* Calcd. for $C_{21}H_{27}NO_5$: C, 67.56; H, 729; N, 3.75. Found: C, 67.62; H, 7.24; N, 4.00.

1-(4′-Methoxybenzyl)-5,6,7-trimethoxy-3,4-dihydroisoquinoline Methiodide (XVI).—The oily dihydroisoquinoline XI (freed from 125 mg. of hydrochloride) in 5 ml. of methanol was refluxed with methyl iodide (3 ml.) on a water bath for 1.5 hr. under nitrogen. The solvent and excess methyl iodide were distilled off under reduced pressure to give a yellowish oily residue which was crystallized from methanol to yield yellow needles (62.3 mg.), m.p. 169–170°. *Anal.* Calcd. for $C_{21}H_{26}INO_4$: C, 52.17; H, 5.38; N, 2.90. Found: C, 52.08; H, 5.51; N, 2.67.

1-(4′-Methoxybenzyl)-5,6,7-trimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline (V). A. From 1-(4′-Methoxybenzyl)-5,6,7-trimethoxy-1,2,3,4-tetrahydroisoquinoline (XII).—A solution of 2.4 g. of XII in 75 ml. of methanol was stirred with 10 ml. of 37% formalin at room temperature for 0.5 hr. Sodium borohydride (3.0 g.) was added and the solution was stirred for an additional 0.5 hr. After the solvent was removed by distillation, the residue was basified with 5% ammonium hydroxide, extracted with ether, and washed with water. The ethereal extract was dried over anhydrous sodium sulfate and evaporated to a yellowish oily substance which was purified by column chromatography on alumina to give an oily base (2.2 g.). The thin layer chromatographic behavior[14] and infrared spectrum of this base were identical with those of an authentic sample of tetrahydrotakatonine. The n.m.r. spectrum[15] (see Figure 1) was identical with that of tetrahydrotakatonine. The hydrochloride had m.p. 188–192°, ultraviolet spectrum λ_{max}^{MeOH} 281 mμ (log ε 3.51). *Anal.* Calcd. for $C_{21}H_{28}ClNO_4$: C, 64.04; H, 7.11; N, 3.55. Found: C, 64.08; H, 7.26; N, 3.56. The picrate had m.p. 143–145°. *Anal.* Calcd. for $C_{27}H_{30}N_{11}O_4$: C, 55.29; H, 5.12; N, 9.56. Found: C, 55.51; H, 5.38; N, 9.42. The melting points of the hydrochloride and picrate were not depressed on admixture with authentic samples of tetrahydrotakatonine hydrochloride and picrate, respectively. Their infrared spectra (KBr) were also superimposable with those of the corresponding salts of tetrahydrotakatonine.

B. From 1-(4′-Methoxybenzyl)-5,6,7-trimethoxy-3,4-dihydroisoquinoline Methiodide (XVI).—To a solution of 400 mg. of XVI in methanol (12 ml.) was added 650 mg. of sodium borohydride during 0.5 hr. with stirring. After stirring for an additional 0.5 hr. at 30–40°, the solvent was distilled off to give a colorless residue which was basified with 5% ammonium hydroxide and extracted with ether. The ethereal extract was washed with water, dried over anhydrous sodium sulfate, and evaporated, to yield an oily residue. The crude residue was purified by column chromatography on alumina to yield a colorless oil. The hydrochloride crystallized as colorless needles (from methanol), m.p. 190–193°; the infrared spectrum (KBr) was identical with that of tetrahydrotakatonine hydrochloride.

1-(4′-Methoxybenzyl)-5,6,7-trimethoxyisoquinoline (XVII).—A solution of 100 mg. of tetrahydroisoquinoline XII in 8 ml. of decalin was refluxed with palladium black at 180–240° for 2 hr. under nitrogen. After cooling, ether was added to the mixture and the catalyst was filtered off. On adding 5% hydrochloric acid, the hydrochloride precipitated. Recrystallization from acetone gave colorless crystals (85 mg.): m.p. 100–105°; ultra-

(14) Thin layer chromatography was carried out with chloroform as solvent on Aluminum Oxide G (Merck).

(15) The n.m.r. spectra were taken on a Varian Associates recording spectrometer (A-60) at 60 Mc. in deuterated chloroform with tetramethylsilane as an internal standard.

violet spectrum λ_{max}^{MeOH} 241.5 mμ (log ε 4.67), 278 (3.70), 325–329 (3.52), and 337 (3.60). *Anal.* Calcd. for $C_{20}H_{23}ClNO_4 \cdot H_2O$: C, 60.86; H, 6.33; N, 3.55. Found: C, 60.26; H, 6.39; N, 3.33.

1-(4'-Methoxybenzyl)-5,6,7-trimethoxyisoquinoline Methiodide (VI).—A solution of 130 mg. of the isoquinoline free base XVII in 4 ml. of methanol was refluxed with methyl iodide (2 ml.) on a water bath for 2.5 hr. under nitrogen. The solvent and excess methyl iodide were evaporated under nitrogen to a yellow residue which was crystallized from methanol to yield yellow plates, m.p. 181–182°, ultraviolet spectrum λ_{max}^{MeOH} 265 mμ (log ε 4.61) and 318 mμ (log ε 3.69). *Anal.* Calcd. for $C_{21}H_{24}INO_4$: C, 52.39; H, 4.99; N, 2.91. Found: C, 52.41; H, 5.26; N, 2.77. The infrared (KBr) and n.m.r. spectra (see Figure 3) were identical with those of an authentic sample of takatonine iodide. The melting point was not depressed upon admixture with the authentic sample of takatonine iodide.

Acknowledgment.—The authors wish to express their gratitude to Dr. T. Shingu for measurements of n.m.r. spectra. They are also grateful to Miss Y. Mano and her collaborators in Kyoto University for elementary analyses. The work at the University of Wisconsin was supported in part by U. S. Public Health Service Research Grant HE-02952, from the National Heart Institute.

Stereochemistry of Enolization of 17-Keto Steroids

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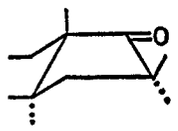
In the enolization of 17-keto steroids the 16α-proton is preferentially removed. Similarly, the protonation of the corresponding enol proceeds preferentially from the α side. This preference is due to steric reasons only.

The influence of steric factors on the course of the enolization–ketonization reaction has been well documented.[1] With cyclic ketones of fixed conformation such as steroid ketones an additional and often overriding factor is stereoelectronic control.[2] Owing to the best orbital overlap in the transition state there is a preference for the removal or addition of the axial proton on the α-carbon in the rate-determining step. In most cases of steroid ketones the steric and stereoelectronic effect are in conflict[2,3] and it is not possible to assess the contribution of each toward the stereochemical course of enolization and ketonization. The C-17 ketone is unique in this respect, since enolization may be expected to be affected by steric factors alone. It has been adequately demonstrated[4,5] that the angle between the plane of the 17-carbonyl and either of the 16α or 16β bonds is identical and hence that ring D in 17-keto steroids is the envelope conformation (A).[5]



A

Since both C-16 bonds have the same degree of axial or equatorial character,[6] no stereoelectronic effect in the enolization of the 17-ketone should exist and preferential removal of either the 16α- or 16β-proton must depend on steric effects alone. The nature and extent of these factors is of additional interest because 17-keto steroid hormones frequently participate in biotransformations which involve reactions of the α- or β-protons of C-16.

The recent successful stereoselective introduction of deuterium into the 16α- and 16β-positions[7] provided the means for preparation of suitable substrates for enolization studies. The use of a hydrogen isotope in this work avoids the obvious objections that may be directed to other C-16 substituents differing greatly from hydrogen in size and electronic character. The stereoselectively 16α- and 16β-tritiated estrone benzoates were prepared by the sequences used in the preparation of the corresponding deuterio compounds[7] in which the orientation of the isotope was confirmed by nmr spectroscopy. Reduction of 16α,17α-epoxyestra-1,3,5(10)-trien-3-ol (I) with lithium aluminum hydride-^3H gave 16β-tritio-17α-estradiol (IIa), which was benzoylated at C-3 and oxidized with the Jones reagent[8] to give the 16β-tritioestrone benzoate (IIIa) (Chart I). The undiminished specific activity of IIIa indicated that the oxidation proceeded without enolization and hence without epimerization. Tritium was introduced into the 16α-position by lithium aluminum hydride reduction of estrone enol diacetate[9] and decomposition of the complex with tritiated acetic acid. The 16α-tritio-17β-estradiol (IVa) thus obtained was benzoylated to IVb and oxidized, also without significant change in specific activity, to 16α-tritioestrone benzoate (IIIb). Based on the deuterium experiments, the stereoselectivity of the tritium introduction was at least 80%, while biochemical evidence[10] supports better than 90% stereoselectivity. The location of the tritium was solely at C-16, since all radioactivity was lost in each instance by exchange in aqueous alkali.

The two epimeric tritiated derivatives IIIa and IIIb were diluted to the same specific activity with inert material and were subjected to acid-catalyzed enolizing

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