Anal. Calcd for $C_8H_{16}O_6S$: C, 39.66; H, 7.49; O, 39.63; S, 13.23. Found: C, 39.62; H, 7.28; O,[21] 39.26; S, 12.99.

This product was identical by mixture melting point and X-ray powder diffraction pattern with the product, mp 177–178°, from the preceding preparation, and with an authentic sample of IV which had been prepared[6] from 1-*S*-ethyl-1-thio-D-galactitol (II) by treatment with aqueous bromine or hydrogen peroxide.

From the zone, R_f 0.14, was obtained D-galactitol (V), 30 mg (2% based on unrecovered starting material), mp 181–183°, identical by mixture melting point and X-ray powder diffraction pattern[22] with an authentic sample of V.

2,3,4,5,6-Penta-*O*-acetyl-1-deoxy-1-ethylsulfinyl-D-galactitol.—Acetic anhydride (1.0 ml) was added to a solution of 1-deoxy-1-ethylsulfinyl-D-galactitol (IV, 125 mg) in pyridine (2 ml), the mixture was kept for 18 hr at room temperature, and ice–water (70 ml) was added. The mixture was extracted with chloroform (two 25-ml portions); the extract was washed with water (two 15-ml portions), dried (magnesium sulfate), and evaporated. The residue was freed from traces of pyridine by codistillation with benzene, and the product was crystallized from benzene (5 ml) and petroleum ether (bp 98–110°, 5 ml): yield 128 mg (55%); mp 146–148°; $[\alpha]^{20}D$ −51 ± 2° (*c* 1.0, chloroform); λ_{max}^{KBr} 5.75 (OAc), 9.45 μ (sulfoxide); λ_{max}^{EtOH} 214 mμ (ε 3800); nmr data (deuteriochloroform), τ 8.68 (three-proton triplet, J = 7.5 cps, CH_3 of ethyl group), 7.86, 7.89, 7.91, 7.98 (singlets, six protons, three protons, three protons, three protons, OAc), 7.28 (two-proton quartet, CH_2 of ethyl group), 7.23 (two-proton doublet, $J_{1,2}$ = 7.0 cps, H-1,1′), 6.15 (one-proton quartet, $J_{5,6}$ = 7.8 cps, $J_{6,6'}$ = 11.6 cps, H-6), 5.69 (one-proton quartet, $J_{5,6'}$ = 4.7 cps, $J_{6,6'}$ = 11.6 cps, H-6′), 4.17–4.42, 4.52–5.80 (one- and three-proton multiplets, H-2,3,4,5); X-ray powder diffraction data, 11.00 m, 8.19 w, 7.01 s (2), 6.46 m, 4.92 vs (1), 4.33 vw, 4.00 w, 3.79 w, 3.67 m, 3.50 m A.

Anal. Calcd for $C_{18}H_{28}O_{11}S$: C, 47.78; H, 6.24; O, 38.90; S, 7.09. Found: C, 47.86; H, 6.51; O,[21] 38.99; S, 6.79.

Irradiation of 1-Deoxy-1-ethylsulfinyl-D-galactitol (IV).—A solution of IV (10 mg) in ethanol (10 ml) was irradiated as described for 8 hr. Crystallization of the resultant dark syrup from ethanol gave galactitol (4 mg, 58%), R_f 0.14, mp 180–183°, undepressed on admixture with authentic galactitol.

Acknowledgments.—The authors thank J. B. Hughes and W. N. Turner for the nmr spectra, Susan J. Gelb for assistance with the chromatographic separations, and H. G. Garg for some of the ultraviolet measurements recorded in Table I. The counsel of Dr. Albert Padwa of this department is gratefully acknowledged.

(21) Determined by Crobaugh Laboratories, Charleston, W. Va.

(22) M. L. Wolfrom and J. N. Schumacher, *J. Am. Chem. Soc.*, **77**, 3318 (1955).

Preparation of Glycosyl Phosphates. β-D-Fructopyranose 2-Phosphate

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A D-fructopyranose 2-phosphate has been prepared by the reaction of the acetylated sugar with anhydrous phosphoric acid. Modifications of the fusion technique using a solvent and using polyphosphoric acid have been investigated, as have been some aspects of the effect of anomeric configuration on the reaction.

The sugar nucleotide "guanosine diphosphate fructose" has been detected in the mold *Eremothecium ashbyii*,[2] and "uridine diphosphate D-fructose" has been isolated from dahlia tubers.[3] The properties of the latter sugar nucleotide were such as to suggest that the D-fructose was present as a glycosyl phosphate, thus making the nucleotide a derivative of D-fructose 2-phosphate. In an effort to gain more insight into this question, a chemical synthesis of such a phosphate was undertaken.

At the time that parts of this work were first reported,[4] chemical syntheses of both D-fructopyranose 2-phosphate and D-fructofuranose 2-phosphate had just been reported by Pontis and Fischer.[5] These workers used a novel synthesis in which the 2-phosphates were prepared from D-fructose 1-phosphate utilizing a carbodiimide-induced cyclization followed by ring opening using alkaline hydrolysis. This procedure, which has also been used to prepare certain aldose 2-phosphates from the corresponding 1-phosphates,[6] gave in low yield either a D-fructopyranose 2-phosphate or a D-fructofuranose 2-phosphate, depending on the conditions used for the cyclization. The products, which were isolated as an amorphous barium salt and as a solution of the sodium salt, respectively, were both assigned the β configuration on the basis of their optical rotations.

In the present paper, the results of some efforts to modify the procedure for glycosyl phosphate formation by fusion are reported, and a simple synthesis of a D-fructopyranose 2-phosphate, using one such modification, is recorded. In its original form,[7] the formation of glycosyl phosphates by the fusion method involved treatment of a fully acetylated reducing sugar (1 mole) with 100% phosphoric acid (4 moles) *in vacuo* at a temperature of 50°, which is just above the melting point of crystalline phosphoric acid. Under these conditions the esters used, namely the penta-*O*-acetates of β-D-glucopyranose and β-D-galactopyranose, dissolved quite readily, and after 2 hr, the reactions were worked up, giving in each instance the α-1-phosphates in purified yields of 30–35%. The procedure was used by Kim and Davidson[8] for the preparation of the α-1-phosphates of 2-acetamido-2-deoxy-α-D-glucopyranose and 2-acetamido-2-deoxy-α-D-galactopyranose from the β-pentaacetates of the corresponding amino sugars. Subsequently, O'Brien[9] carried out a fusion on 2-amino-2-deoxy-α-D-glucopyranose pentaacetate and, by ion-exchange chromatography, was able to isolate both the α and β anomer of the 1-phosphate formed in the reaction. The conditions

(1) Research Career Development Awardee, U. S. Public Health Service. This work was also supported by Public Health Service Grant GM 09756.

(2) H. G. Pontis, A. L. James, and J. Baddiley, *Biochem. J.*, **75**, 428 (1960).

(3) N. S. González and H. G. Pontis, *Biochim. Biophys. Acta*, **69**, 179 (1963).

(4) D. L. MacDonald, Abstracts, 147th National Meeting of the American Chemical Society, Philadelphia, Pa., April 1964, p 1C.

(5) H. G. Pontis and C. L. Fischer, *Biochem. J.*, **89**, 452 (1963).

(6) R. Piras, *Arch. Biochem. Biophys.*, **103**, 291 (1963).

(7) D. L. MacDonald, *J. Org. Chem.*, **27**, 1107 (1962).

(8) T. Y. Kim and E. A. Davidson, *ibid.*, **28**, 2475 (1963).

(9) P. J. O'Brien, *Biochim. Biophys. Acta*, **86**, 628 (1964).

useful for the preparation of 1-phosphates from acetylated reducing sugars with the 1,2-*trans*-diequatorial configuration, of which the β-D-*gluco* and β-D-*galacto* configurations are examples, were found by O'Brien[9] to be unsuitable for reactions involving sugars with 1,2-*cis* configuration. Thus, 2-amino-2-deoxy-α-D-glucopyranose pentaacetate, where the 1,2-*cis* configuration makes difficult the displacement of acetate on the 1-carbon by neighboring-group participation of the acetamido group on the 2-carbon,[10] did not react well at 50°. Crude yields of up to 33% were obtained, however, when the reaction was run at 83° for 45 min, using a molar ratio of phosphoric acid to sugar acetate to 8:1. Recently, it has been shown that reaction of α-D-glucopyranose pentaacetate with phosphoric acid at 85° for 45 min gives α-D-glucopyranose 1-phosphate in a purified yield of 27% (crude yield 40%);[11] when the reaction was run at 50° for 2 hr, the crude yield of acid-labile phosphate was only 10%.[12] The fusion of α-D-galactopyranose pentaacetate at 85° for 45 min is described below. The product isolated, α-D-galactopyranose 1-phosphate, is accompanied by considerable amounts of decomposition products which make difficult the isolation of the material by direct crystallization.[13] The final yield of almost pure α-1-phosphate is only about 20%. By contrast, compounds with 1,2-*trans*-diequatorial configuration react very well. We have recently improved the original procedure with such compounds, so that by using a molar ratio of phosphoric acid to sugar acetate of 8:1, with stirring for 2 hr at 50° *in vacuo*, one can obtain the potassium salts of α-D-glucopyranose 1-phosphate and α-D-galactopyranose 1-phosphate from the β-pentaacetates of the corresponding sugars in purified yields of about 60%.[11] Under these conditions tetra-*O*-acetyl-β-D-ribofuranose gave 30% of an as yet uncharacterized product.

Certain ketose phosphates can be prepared by this general fusion technique. For example, β-D-fructopyranose pentaacetate reacted rapidly and with considerable decomposition with phosphoric acid at 50°. At about 30°, using molar ratios of phosphoric acid to sugar acetate of 4:1 or 8:1, acid-labile phosphate was formed in yields of 25–40%, in 1.5 hr, and a D-fructopyranose 2-phosphate could be isolated as a crystalline cyclohexylammonium salt in yields of 20–30%. This salt proved identical with the one prepared by a different method described below. Of interest is the observation that in the reactions of β-D-fructopyranose pentaacetate with phosphoric acid, where the molar ratio of phosphoric acid to sugar acetate is 40:1, the yields of acid-labile phosphate drop to 4–5%. Under all conditions tried, namely room temperature, 50, and 85°, with molar ratios of phosphoric acid to sugar acetate of 4:1 or 8:1 for 1.5 hr, D-fructofuranose pentaacetate[14] gave acid-labile phosphate in yields of only 3–5%. While working with this phosphate, we attempted to purify the product on a column of Dowex 1 × 4 (200–400 mesh), borate form, using a linear gradient of ammonium borate, following the conditions of Pontis and Fischer.[5] These authors reported that both D-fructofuranose 2-phosphate and D-fructopyranose 2-phosphate were eluted by ammonium borate concentrations below about 0.3 *M*, pH 7.4. In our hands D-fructopyranose 2-phosphate was not eluted with 0.5 *M* ammonium borate, pH 7.4; it was eluted readily with 0.2 *M* potassium chloride. Further experimentation will be necessary to resolve this discrepancy.

Using mild conditions, attempts were made to prepare glycosyl phosphates from certain other sugar derivatives, in particular the glycosides. In general, the crude yields of acid-labile phosphate discouraged further experimentation. Methyl α-D-glucopyranoside tetraacetate and methyl β-D-ribofuranoside triacetate each gave about 9% in 7 hr at 50°, and methyl α-D-fructofuranoside tetraacetate gave only about 0.4% in 5 hr at room temperature and 5% in 2 hr at 50°. Doubtless, glycosyl phosphates could be isolated from these mixtures, but our experiments were confined to alkaline hydrolysis followed by phosphate analysis and paper chromatographic examination, which revealed the presence of phosphorylated sugars of the anticipated acid lability.

Crystalline phosphoric acid, on being melted, is converted rapidly into a mixture of ortho- and pyrophosphoric acids with concomitant formation of water; the product contains approximately 6 mole % of water.[15] Reasoning that this water may influence the course of the reaction of an acetylated sugar with phosphoric acid, we tried "105% phosphoric acid."[16] This material contains appreciable amounts of pyrophosphoric acid (28 mole %) as well as lesser amounts of triphosphate and tetraphosphate, and about 2 mole % of water.[15] As described below, it was allowed to react at room temperature with β-D-glucopyranose pentaacetate. This was followed by alkaline hydrolysis for several hours on the steam bath to hydrolyze the oligophosphates, and from this mixture α-D-glucopyranose 1-phosphate, purified *via* the cyclohexylammonium salt, was isolated as the potassium salt in 46% yield. Thus, the nature of the product and its yield are about the same as when using 100% phosphoric acid. Analogous to its behavior toward 100% phosphoric acid, α-D-glucopyranose pentaacetate reacted very sluggishly with "105% phosphoric acid"; in 45 min at 85°, the yield of acid-labile phosphate was only 6%. Penta-*O*-acetyl-β-D-fructopyranose decomposes quite rapidly in "105% phosphoric acid," and yields of acid-labile phosphate are invariably low (1–3%) when the reaction is run at room temperature for 5 min or 1 hr, with varying ratios of phosphorylating agent to sugar acetate. On the other hand, β-D-ribofuranose tetraacetate gave about 40% of acid-labile phosphate; the products of this reaction are currently under investigation.

Finally, the use of 100% phosphoric acid in a nonpolar solvent was investigated and tetrahydrofuran

(10) Some aspects of replacement reactions at the 1-carbon are reviewed by R. U. Lemieux, *Advan. Carbohydrate Chem.*, **9**, 1 (1954).

(11) D. L. MacDonald in "Methods in Enzymology," Academic Press Inc., New York, N. Y., in press.

(12) Dr. E. A. Davidson (personal communication) has reported similar results when the reaction was run under these conditions.

(13) Mr. Roger Wong, working in these laboratories, has shown that reactions carried out at these higher temperatures may lead to the release of more than the theoretical amount of volatile acid, as determined by titration. As yet, however, we have no evidence of the replacement of acetoxy by phosphate at any position other than the anomeric carbon atom.

(14) R. Kuhn and H. Grassner, *Ann.*, **610**, 122 (1957).

(15) A.-L. Huhti and P. A. Gartaganis, *Can. J. Chem.*, **34**, 785 (1956).

(16) We wish to thank Dr. Allan K. Lazarus of the FMC Corp., New York, N. Y., for a sample of their 105% phosphoric acid, and for helpful suggestions concerning its use.

was found to be a suitable medium for certain reactions. A reaction time of 2 hr and a temperature of 55° gave a mixture from which β-D-fructopyranose 1-phosphate could be isolated in 54% yield from β-D-fructopyranose pentaacetate. Under the same conditions, β-D-glucopyranose pentaacetate reacted to the extent of only 0.2% and D-fructofuranose pentaacetate gave 1.1% in 2 hr and 2.2% in 24 hr. Some of the results using β-D-fructopyranose pentaacetate are shown in Table I, and they illustrate the critical dependence of yield on the solvent and on the proportions of the reactants used.

TABLE I

REACTIONS OF β-D-FRUCTOPYRANOSE PENTAACETATE WITH H_3PO_4 IN SOLVENT

H_3PO_4, g	Sugar Acs, g	Solvent (ml)	Time, hr	Temp, °C	Crude yield, %
5	5	THF[a] (5)	1	50	42
5	5	THF (5)	2	50	48
5	5	THF (5)	12	50	34
5	5	THF (5)	2	55	56
1	1	THF (5)	2	50	0.3
5	1	THF (5)	2	50	12
5	5	DMF[a] (5)	24	50	0.6
5	5	DMSO[a] (5)	24	50	0.08

[a] THF, tetrahydrofuran; DMF, dimethylformamide; DMSO, dimethyl sulfoxide. The acetylated sugar had not dissolved completely in the DMF or DMSO in the time indicated.

In the present work, the same cyclohexylammonium D-fructopyranose 2-phosphate was isolated whether it was formed in the presence or absence of a solvent. The compound had a rotation of −77.9° in water. This corresponds to a molecular rotation of −35,700, a value which agrees well with the value −32,900 found by Pontis and Fischer[5] for the barium salt of D-fructopyranose 2-phosphate which they obtained from D-fructose 1-phosphate by phosphate migration. On the basis of a comparison of the rotation of their phosphate with that of known α- and β-D-fructopyranosides, these authors concluded the compound was β-D-fructopyranose 2-phosphate, a conclusion which appears valid in the absence of any conflicting evidence.

Pontis and Fischer[5] determined the acid lability of their β-D-fructopyranose 2-phosphate and found at pH 4 and 37° a half-life of 28 min. With the larger quantity of material made available by the synthesis described in the present paper, we used slightly different assay conditions; at pH 4 and 37° the first-order reaction constant, 3.4×10^{-2} min^{-1} corresponds to a half-life of 20 min.

Experimental Section

Di(cyclohexylammonium) β-D-Fructopyranose 2-Phosphate.—Five grams of crystalline phosphoric acid,[17] which had been dried overnight at room temperature *in vacuo* over magnesium perchlorate, was dissolved in 5 ml of tetrahydrofuran (distilled from lithium aluminum hydride and stored over Linde Molecular Sieves, Type 3A). To the solution was added 5.00 g (12.8 mmoles) of powdered β-D-fructopyranose pentaacetate[18] and the stoppered mixture was placed in a water bath at 55° and stirred magnetically. The acetate dissolved in a period of about 0.75 hr, and after a total of 2 hr, the light brown solution was cooled in ice and to it was added 120 ml of ice-cold 2 *N* lithium hydroxide. The mixture was shaken immediately and then set aside overnight at room temperature. The precipitated lithium phosphate was removed by filtration through Celite and washed with dilute lithium hydroxide (*ca.* 0.01 *N*). The clear amber solution contained 7.2 mmoles (56%) of phosphate.[19]

The pH of the solution was lowered to about 8.6 by addition of Dowex 50W-H^+ and, after the resin had been removed by filtration, barium acetate (3.8 g) was added and the solution was concentrated under reduced pressure to about 35 ml. Traces of insoluble matter were removed by centrifugation, and the barium salt was precipitated by the addition of three volumes of ethanol. After several hours at 5°, the salt was collected by centrifugation, washed in turn with 95% alcohol, acetone, and ether, and air dried. The salt was dissolved in 35 ml of water and the solution was freed if necessary of insoluble matter by centrifugation. The salt was reprecipitated as above and after a third such precipitation, it was converted to the cyclohexylammonium form by dissolution in water followed by passage of the solution through a column of Dowex 50W in the cyclohexylammonium form (1.5 × 28 cm). The column was washed with 150 ml of water and the effluent was then treated with charcoal, filtered, and concentrated *in vacuo* (bath 35°). The residue was crystallized from water (20 ml) by the addition over a period of 2 hr of ten parts of acetone; the salt was collected after 16 hr at 5° and air dried: 3.18 g, $[\alpha]^{24}D$ −77.7° (*c* 1, water). Recrystallization in the same manner gave 3.11 g (6.8 mmoles, 53%), $[\alpha]^{24}D$ −77.9°.

Anal.[20] Calcd for $C_{18}H_{39}N_2O_9P$ (458.5): C, 47.20; H, 8.58; N, 6.12; P, 6.77. Found: C, 47.24; H, 8.80; N, 6.16; P, 6.75.

For **acid hydrolysis,** 57.4 mg of the salt was dissolved in 5 ml of 1 *M* acetate buffer, pH 4.0, and the solution was placed in a water bath at 37°. Samples (0.1 ml) were transferred at intervals to test tubes containing 1.0 ml of magnesia mixture (2 *M* in ammonium hydroxide, 0.05 *M* in magnesium acetate, and 0.5 *M* in ammonium chloride). The tubes were set at 0° overnight and then centrifuged and duplicate 0.1-ml samples of the supernatant were withdrawn for phosphate determination.[19]

Dipotassium α-D-Glucopyranose 1-Phosphate, Using 105% Phosphoric Acid.—A sample of β-D-glucopyranose pentaacetate (5.00 g, 12.8 mmoles) was stirred magnetically *in vacuo* (oil pump) with 10 ml of 105% phosphoric acid[16] at room temperature for 2 hr, following which 330 ml of ice-cold 2 *N* lithium hydroxide was added. The mixture was shaken thoroughly to ensure complete dispersal of the syrup, and it was then heated on the steam bath for 4 hr to hydrolyze polyphosphates. The basic solution was cooled and the precipitated lithium phosphate was removed by filtration through Celite and washed with cold lithium hydroxide (*ca.* 0.01 *N*). The resulting brown solution, which contained 6.75 mmoles of phosphate (52%), was cooled to *ca.* 5° and passed through a precooled column of Dowex 50W-H^+ (1.9 × 30 cm) and the column was washed with 250 ml of water. The effluent was collected in water containing 10 ml of cyclohexylamine. The colorless solution which remained after charcoal treatment was concentrated *in vacuo*, and the resulting syrup was dissolved in 100 ml of warm absolute alcohol. Crystallization, which began soon at room temperature, was complete after 2 days at 5°. The cyclohexylammonium salt obtained (2.9 g, $[\alpha]^{26}D$ +59° in water) was dissolved in water containing 1 g of potassium hydroxide and the solution was concentrated under reduced pressure. Removal of cyclohexylamine was completed by adding 50 ml of water and removing it *in vacuo* and repeating the process. The residue was dissolved in 20 ml of water, excess potassium hydroxide was removed by the addition of Dowex 50W-H^+ to bring the pH to 9, and the resin was removed by filtration and washed with water (5 ml). Careful addition of three volumes of absolute alcohol gave dipotassium α-D-glucopyranose 1-phosphate dihydrate which, after drying over calcium chloride at 15 mm, weighed 2.19 g (5.9 mmoles, 46%) and showed $[\alpha]^{25}D$ +77.3° (*c* 2, water), lit.[21] $[\alpha]^{25}D$ +78°.

Reaction of α-D-Galactopyranose Pentaacetate with Anhydrous Phosphoric Acid.—A sample of α-D-galactopyranose penta-

(17) The grade used was of a stated purity, ≥99%, purchased from Fluka AG, Buchs, S.G., Switzerland.

(18) C. S. Hudson and F. Brauns, *J. Am. Chem. Soc.*, **37**, 1283 (1915).

(19) G. R. Bartlett, *J. Biol. Chem.*, **234**, 466 (1959).

(20) Analyses were performed by Elek Microanalytical Laboratories, Torrance, Calif.

(21) M. L. Wolfrom and D. E. Pletcher, *J. Am. Chem. Soc.*, **63**, 1050 (1941).

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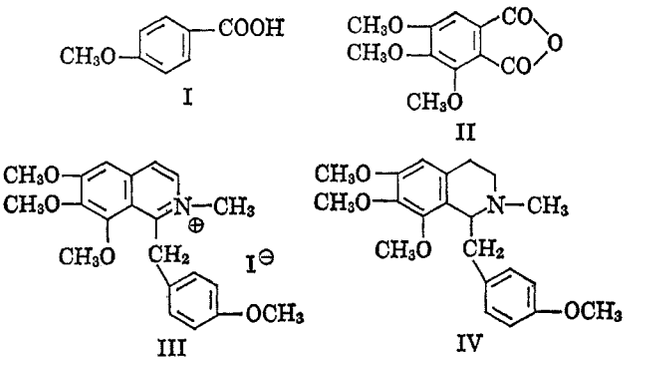
Total syntheses of takatonine iodide and tetrahydrotakatonine by unequivocal routes show that takatonine iodide should be assigned the 1-(4′-methoxybenzyl)-5,6,7-trimethoxyisoquinoline methiodide structure (VI), rather than the isomeric 6,7,8-trimethoxy structure III considered earlier.

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).