Urea.—Mallinckrodt Analytical Reagent Grade crystalline urea was used without further purification.

Monoacetyl α-Chymotrypsin (AC-A).—The isolation procedure of Balls and Wood[3] was used with certain modifications. The adsorption step employing acid washed charcoal was eliminated. With certain lots of α-chymotrypsin, the unique spectral properties of this intermediate did not appear after 45 minutes incubation at room temperature. In those cases, the incubation times were extended until aliquots of the reaction mixture, after extraction of excess reagent and *p*-nitrophenol, showed the increase in absorbancy at 245 mμ upon raising the *p*H.

Monoacetyl-α-chymotrypsin (AC-I).—The method of preparation of this intermediate was identical to the method above, except that the time of incubation of chymotrypsin with *p*-nitrophenyl acetate was reduced to ten minutes at room temperature. An increase in absorbancy at 245 mμ was not observed when solutions of AC-I were adjusted to *p*H 8.0.

Determination of Extent of Acylation.—Complete acetylation of the monoacetyl α-chymotrypsin preparations used was ascertained both by spectrophotometric methods[22] and by reaction with hydroxylamine.

Treated α-Chymotrypsin (T-CT).—Solutions of α-chymotrypsin were treated in a manner identical to the preparation of AC-A, except for the exclusion of *p*-nitrophenyl acetate.

Methods. Instrument.—A Cary Model 14 self-recording spectrophotometer was employed for all kinetic and spectral studies. Temperature control (±0.5°) was effected by the use of a water jacketed cell holder.

Kinetic Studies.—Unless otherwise indicated, water solutions of 6 mg. per ml. of enzyme by weight were filtered through Whatman 42 filter paper and kept on ice until used. Before use, aliquots of these solutions were preincubated at the reaction temperature, usually 25 ± 0.5°. A 1.0 ml. aliquot of enzyme solution was introduced into the sample cuvette, and, at zero time, 2.0 ml. of 0.05 *M* Tris–HCl buffer, which was also 0.05 *M* with respect to calcium chloride, was added with a syringe type mixing device. Good mixing was obtained and readings of absorbancy at 245 mμ were begun within a maximum time of five seconds. Water was used as the reference solution. Apparent first order rate constants for the decay of absorbancy at 245 mμ were estimated from the slopes of plots of $-\ln(\Delta D)$ *versus* time in seconds, where ΔD is the difference between absorbancy at time t and the final absorbancy. Apparent first order rate constants for the increase in absorbancy were estimated from plots of $-\ln(-\Delta D)$ *versus* time in seconds, where ΔD was the difference between absorbancy at time t and the maximum absorbancy.

Effect of Urea on Absorbancy.—Water solutions of 60 mg./ml. of enzyme by weight were prepared as needed. Aliquots of 0.10 ml. were pipetted into each of two cuvettes. 0.20 ml. of 0.05 *M* Tris was added to adjust the *p*H to 8.0. To one cuvette 2.7 ml. of 8.9 *M* urea in 0.05 *M* Tris–HCl at *p*H 8.0, were added after a suitable interval to allow for increase in absorbancy. The other sample was allowed to deacylate for 1 hr., and than an equal amount of buffered urea was introduced. The absorbancy of these solutions at 245 mμ was compared to a reference solution of 8 *M* urea at various time intervals. Control solutions were compared in which the urea solution was replaced with aqueous buffer to insure that, at the initial high enzyme concentration, the increase and subsequent decay in absorbancy occurred normally in the absence of urea.

Difference Spectra.—Water solutions usually containing 3 mg. per ml. of enzyme by weight were prepared, filtered through Whatman #42 filter paper and kept on ice until needed. A 1.0 ml. aliquot of this solution was pipetted into the reference cuvette and 2.0 ml. of 0.05 *M* Tris–HCl, 0.05 *M* calcium chloride buffer at the desired *p*H were added and the sample allowed to deacylate. Then sufficient dilute HCl was added with a micropipet to bring the solution to the desired final *p*H and the solution was mixed. This constituted the reference solution. Unless otherwise indicated, for the sample solution 2.0 ml. of buffer and the desired amount of acid were placed in the cuvette. The enzyme solution was added and the solution mixed. For each pair of samples, the same pipette was used to add each solution, and extreme care was exercised to prevent concentration discrepancies. Gravimetric trial experiments indicated that total pipetting errors could be kept within limits such that, at an optical density of 2.0 at 280 mμ, the two solutions would not differ by more than an optical density of 0.003. Silicone coated pipets were used for addition of buffer. For introduction of enzyme solutions, however, acid cleaned transfer pipets were found to be far superior in reproducibility.

The difference spectra were scanned from 320 to 230 mμ at a scanning speed of 2.5 ångströms per second. At *p*H's at which absorbancy was changing, the scanning speed was increased to 10 Angstroms per second, and smaller wave length regions of particular interest were covered.

Below 240 mμ the slit width was extremely wide (1 to 3 mm.) and changing rapidly. Therefore, at the enzyme concentrations used, any spectral findings in this region must be viewed with skepticism. The maximum slit width observed in the 245–320 region was 0.2 mm.

Determination of Protein Concentration.—Protein concentrations were determined spectrophotometrically at 280 mμ by using $E^{280}_{1\%} = 20.0$ to relate extinction to protein concentration.[22] The molecular weight of α-chymotrypsin was taken as 25,000.

Determination of Extent of Acylation.—The extent of acylation of the enzyme was ascertained by reaction with hydroxylamine. A slight modification[9] of the procedure of Hestrin was used.[26]

(26) S. Hestrin, *J. Biol. Chem.*, **180**, 249 (1949).

[CONTRIBUTION FROM THE RESEARCH DEPARTMENT, CIBA PHARMACEUTICAL PRODUCTS, INC., SUMMIT, N. J.]

Rauwolfia Alkaloids. XXXIX. Methyl Neoreserpate, an Isomer of Methyl Reserpate. Part 2. Mechanism of Formation

BY WILLIAM E. ROSEN AND HERBERT SHEPPARD

RECEIVED MAY 15, 1961

The equilibrium ratio of methyl reserpate (I) to methyl neoreserpate (II) has been found to be approximately 1:5. The mechanism of the conversion of I to II has been studied using methanol-C^{14}, and the results support the methanol elimination, methanol addition scheme previously proposed.

In our previous paper,[1] the formation of methyl neoreserpate from methyl reserpate by treatment with refluxing methanolic sodium methoxide was reported. Structure II was assigned to methyl neoreserpate on the basis of chemical conversions. In the present paper, the equilibrium between the two isomers is examined, and experiments using methanol-C^{14} are described which support the mechanism previously proposed.[1]

The ratio of methyl reserpate (I) to methyl neoreserpate (II) at equilibrium was found to be approximately 1:5. This ratio represents a free energy difference of 1–1.5 kcal./mole in favor of II.[2] Table I lists the ratios of I:II found after re-

(1) W. E. Rosen and J. M. O Connor, *J. Org. Chem.*, **26**, 3051 (1961).

(2) A discussion of stabilities based on conformational considerations was presented in Part 1.[1]

fluxing I or II with methanolic sodium methoxide.[3] The long refluxing times needed to reach equilibrium indicate a considerable energy barrier between the two isomers.

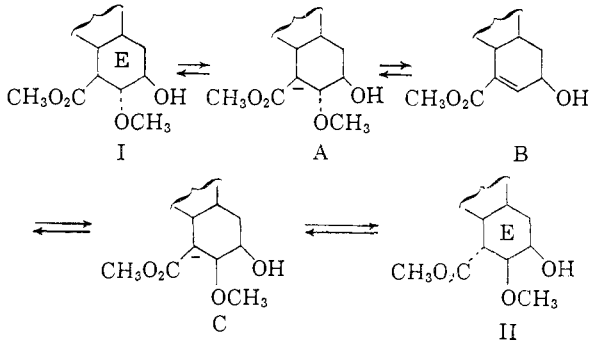
TABLE I

COMPOSITION OF EQUILIBRIUM MIXTURES OF METHYL RESERPATE (I) AND METHYL NEORESERPATE (II) OBTAINED BY REFLUXING EITHER I OR II WITH METHANOLIC SODIUM METHOXIDE

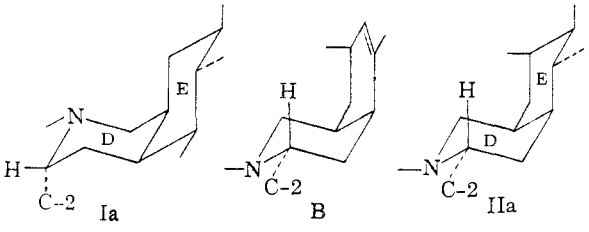
Starting material	Reflux time[a]	Estd. ratio I:II[b]
I	4 hr.	99:1
I	21 hr.	4:1
I	64 hr.	1:2
I	14 days	1:5
II	64 hr.	1:8
II	14 days	1:5

[a] Extending the refluxing time gave increasing amounts of by-products. The recovery of I plus II was only *ca.* 20% after 14 days. [b] The amounts were estimated by paper chromatographic comparison with standards.

The mechanism previously suggested[1] for the equilibrium involved the elimination and re-addition of methanol.



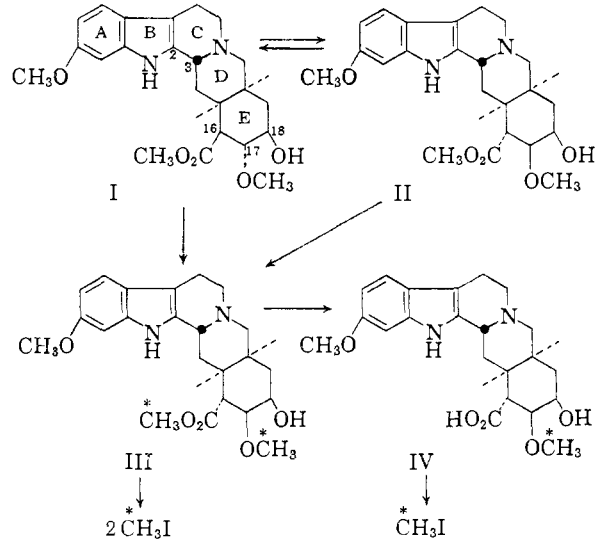
We picture the free energy curve of this reaction as four-peaked, with intermediates A, B and C in the energy troughs between I and II. The likely conformations of methyl reserpate, the intermediate B, and methyl neoreserpate (see partial structures Ia, B, and IIa, respectively) suggest that fewer changes in the positions of atoms are required to go from II to B (or B to II) than are



required to go from I to B (or B to I). The consequence of such a situation is that the energy barrier between I and B is greater than that between II and B. Although the intermediate B has not been isolated, this conclusion is supported by the experiments with methanol-C^{14} described below.

The mechanism proposed for conversion of I to II requires that the methoxyl group at C-17 become equilibrated with the methanol solvent. The methoxyl group of the C-16 carbomethoxyl function should also equilibrate with solvent during the reaction. When the conversion of I to II was carried out in methanol-C^{14} (see Table II in Experimental), the isolated methyl neoreserpate (III) was indeed found to contain *two* equilibrated methoxyl groups. Cleavage of the methoxyl groups of III with hydriodic acid[4] gave methyl iodide-C^{14} (see Table III) and left an alkaloid fragment which contained no C^{14}-activity. When unlabeled methyl neoreserpate (II) was refluxed with sodium methoxide in methanol-C^{14}, the recovered methyl neoreserpate (III) was also found to have incorporated two[5] atoms of C^{14}.

When methyl neoreserpate-C^{14} was converted to neoreserpic acid-C^{14} (IV) by refluxing for 4 hours with methanolic potassium hydroxide, the label at C-16 was removed completely, and approximately 80% of the C^{14}-activity at C-17 was lost. The C-17 methoxyl group had evidently become equilibrated to a considerable extent with the methanol, probably through an intermediate analogous to B, yet the isolated IV contained only about 5% reserpic acid contamination. Cleavage of the methoxyl group of IV gave methyl iodide-C^{14}.



When methyl neoreserpate-C^{14} was refluxed[6] for 4 hours with methanolic sodium methoxide, 67% of the C^{14}-activity was lost. If the methoxyl groups at C-16 and C-17 had been equally equilibrated with solvent, the loss of C^{14}-activity at C-17 would be 67%. If C-16 had lost *all* of its activity by complete equilibration with solvent, the C^{14} loss at C-17, necessarily through intermediate B, would be 33%. Yet paper chromatography showed that the resultant methyl neoreserpate-C^{14} contained only 5–10% methyl reserpate. The only way that methyl neoreserpate could be 33–

(3) In the absence of sodium methoxide, methyl reserpate was completely unaffected by refluxing for 4 days in methanol.

(4) J. B. Niederl and V. Niederl, "Organic Quantitative Microanalysis," Second Edition, John Wiley and Sons, Inc., 1942, pp. 240–244.

(5) Methyl neoreserpate (II) did not dissolve completely in the reaction mixture during its conversion to III (see Experimental section). This insolubility may account for the slightly lower C^{14}-activity of the isolated III (see Table II).

(6) The following discussion assumes that starting methyl neoreserpate-C^{14} was equally labeled at C-16 and C-17.

TABLE II
EXCHANGE OF METHYL GROUPS USING METHANOL-C^{14}[a]

	Sample counted (wt. in mg.)		Source, methyl	D.p.m.	Specific activity, $\times 10^{-4}$ μc./μM.	Calcd. no. of labelled methyl groups[b]
1[f]	Methyl neoreserpate	(1.000)	Reserpate	2060	4.30	1.82
		(1.000)		1955	4.25	1.80
2[f]	Methyl neoreserpate[c]	(1.084)	Reserpate	2030	4.00	1.70
		(1.158)		1920	4.10	1.74
		(1.027)		2070	4.32	1.83
		(1.032)		2040	4.28	1.81
3	Methyl neoreserpate[d]	(1.000)	Neoreserpate[d]	1820	3.89	1.65
		(1.000)		1720	3.67	1.56
4	Methyl neoreserpate[e]	(4.126)	Neoreserpate[e,g]	2750	1.24	0.53
5	Neoreserpic acid[c]	(1.255)	Neoreserpate[h]	244	0.39	0.165
		(4.944)		918	0.37	0.157
		(4.913)		890	0.36	0.152

[a] Methanol-C^{14} (from New England Nuclear Corp., Boston, Mass.) having a specific activity of 0.115 μc./μM. (estimated purity, 95%) was diluted with anhydrous methanol to a specific activity of 2.36×10^{-4} μc./μM. for these experiments. [b] Calculated by dividing the found specific activity by the specific activity of the methanol-C^{14} (2.36×10^{-4} μc./μM.). [c] See experimental details below. [d] Unlabeled methyl neoreserpate was refluxed for 64 hours in methanolic-C^{14} sodium methoxide (see footnote 5). [e] C^{14}-labeled sample 3 was refluxed for 4 hours in unlabeled methanolic sodium methoxide. [f] 1 and 2 are duplicate runs. [g] Sample 3. [h] Sample 2.

67% equilibrated with intermediate B, while only 5–10% equilibrated with methyl reserpate, is for the energy barrier between II and B to be lower than that between I and B. A possible explanation for the difference was suggested above.

Acknowledgment.—The authors thank the following people for their contributions to this work: Mr. B. P. Korzun and Mr. S. M. Brody for the paper chromatographic determinations; Mr. L. Dorfman and his associates for the microanalytical determinations, especially Mr. G. E. Robertson for cleavage of methoxyl and capture of methyl iodide; Dr. C. R. Rehm for the gas–liquid chromatography determination; and Mr. J. M. O'Connor and Mr. T. F. Mowles for technical assistance. We thank Dr. A. C. Shabica for his encouragement of this work.

Experimental[7]

Paper Chromatography.[8]—Mixtures of methyl reserpate and methyl neoreserpate were chromatographed in at least two systems for comparison with standard mixtures. The most satisfactory system employed Whatman No. 1 paper impregnated with formamide (adjusted to *p*H 5.6 with benzoic acid), and a chloroform-mobile phase. In this system, methyl reserpate gave a spot at R_f 0.14 which had a yellowish-green fluorescence in ultraviolet light, and methyl neoreserpate gave a spot at R_f 0.18 which had a bluish fluorescence in ultraviolet light. Neoreserpic acid was chromatographed on untreated Whatman No. 1 paper using 1-butanol saturated with 0.1 *N* ammonium hydroxide as mobile phase; in this system neoreserpic acid had R_f 0.22 and reserpic acid had R_f 0.45.

Carbon-14 Analyses.—All samples were counted in a Packard Tri-Carb liquid scintillation spectrometer in 10 ml. of toluene. The solutions contained 0.3% 2,5-diphenyloxazole (PPO) and 0.01% 1,4-bis-2(5-phenyloxazolyl)-benzene (POPOP) as phosphors. Neoreserpic acid samples were first dissolved in 1 ml. of absolute ethanol and then diluted with 10 ml. of toluene–phosphor solution. By choosing appropriate sample sizes and counting times, the errors in counting were kept below 5% in all cases. Following the initial count, 0.1 ml. of a standard toluene-C^{14} solution was added and the solutions were recounted, in order to determine the counting efficiency. Using the counting efficiency as determined for each solution, the observed count rate was converted to the absolute count rate, *i.e.*, disintegrations per minute (d.p.m.).

(7) Melting points were taken on an electrically-heated aluminum block and are uncorrected.

(8) B. P. Korzun and S. M. Brody, *J. Chromatog.*, in press.

TABLE III
C^{14}-CONTENT OF METHYL IODIDE OBTAINED FROM METHYL NEORESERPATE-C^{14} AND NEORESERPIC ACID-C^{14}

Starting material			D.p.m. of methyl iodide collected[a]	Recovery, %
Source	Wt., mg.	D.p.m.		
Methyl neoreserpate-C^{14}	4.37	8210	6360	77.5
(see Table II, 2)	1.40	2630	2040	77.6
Neoreserpic acid-C^{14}	4.93	923	576	62.4
(see Table II, 5)	4.68	876	467	53.4

[a] The methyl iodide-C^{14} was captured in toluene at 0–5° (contact time approximately 1 second), instead of in sodium acetate and bromine in glacial acetic acid,[4] in order to allow C^{14} counting in the Packard Tri-Carb liquid scintillation spectrometer. When a control run was carried out under the same conditions with an analytical sample of methyl neoreserpate, where the amount of methyl iodide generated was known, the cold toluene captured 74% of the methyl iodide. The captured methyl iodide was measured by gas–liquid chromatography; quantitative determination of methyl iodide concentration was made by peak area measurement of the sample and comparison with a reference standard of methyl iodide.

Preparation of Methyl Neoreserpate-C^{14} (III) from Methyl Reserpate (I).—A solution of 1.20 g. of commercial sodium methoxide in 100 ml. of methanol-C^{14} (specific activity, 2.36×10^{-4} μc./μM.) was stirred 4 hours at room temperature under a drying tube. After 6.67 g. of methyl reserpate (I) had been added, the mixture was refluxed for 64 hours with stirring. The yellow solution was diluted with 100 ml. of water (containing 3.5 g. of sodium chloride) and extracted with three 50-ml. portions of methylene chloride. The combined extract was washed once with 50 ml. of water and once with 50 ml. of saturated sodium chloride solution, and then was dried over anhydrous sodium sulfate. The filtered solution was concentrated to 23 ml., seeded, and chilled at −5° for 4 hours. The white crystals were collected and dried, giving 1.73 g.; the mother liquors were concentrated to 5 ml., giving a second crop of 0.40 g. The combined crops were recrystallized from benzene–isopropyl alcohol, giving 1.97 g. (25.8%) of white needles of methyl neoreserpate-C^{14} (III) as a monoisopropanol solvate, m.p. 145–148°, $[\alpha]^{22}D$ +49.7° ($CHCl_3$). The specific activity was 4.2×10^{-4} μc./μM.

Anal. Calcd. for $C_{23}H_{30}N_2O_5 \cdot C_3H_8O$ (474.61): C, 65.80; H, 8.07; N, 5.90. Found: C, 66.03; H, 8.21; N, 6.21.

Preparation of Methyl Neoreserpate-C^{14} (III) from Methyl Neoreserpate (II).—A solution of 0.18 g. of sodium methoxide in 15.0 ml. of methanol-C^{14} (specific activity, 2.36×10^{-4} μc./μM.) was stirred 20 minutes at room temperature and 1.15 g. of methyl neoreserpate (II) mono-2-propanol

solvate[1] was added. The suspension was stirred and refluxed for 64 hours under a drying tube without becoming a clear solution. The suspension was worked up in the same way as the reaction mixture from methyl reserpate, giving 0.39 g. of white crystals from methylene chloride, and 0.25 g. (21.8%) of white needles, m.p. 146–149°, after recrystallization from benzene–isopropyl alcohol. Its specific activity was 3.8×10^{-4} μc./μM.

Preparation of Neoreserpic Acid-C^{14} (IV) as its Hydrochloride Salt from III.—A cloudy solution of 1.80 g. of methyl neoreserpate-C^{14} (III) monoisopropanol solvate (specific activity, 4.2 μc./μM.) and 7.2 g. of potassium hydroxide in 108 ml. of methanol was stirred and refluxed for 4 hours. After being filtered, the solution was stripped to dryness at reduced pressure, and the residue was taken up in 36 ml. of water and washed four times with 18-ml. portions of chloroform. Water was removed at reduced pressure and the residue was taken up in 32 ml. of methanol, acidified by addition of 10 ml. of concentrated hydrochloric acid (36%) and filtered; the insoluble potassium chloride was washed with 14 ml. of chloroform–methanol (4:1). The combined filtrate and wash was stripped to dryness, taken up in 72 ml. of chloroform–methanol (4:3), and passed through a bed of Darco G-60. The filtrate was stripped to dryness, taken up in 50 ml. of chloroform–methanol (4:1), and passed again through a bed of Darco G-60; the Darco was washed with 25 ml. of fresh solvent mixture. Removal of solvent left 1.40 g. of yellow solid. Two precipitations from 15 ml. of methanol and 150 ml. of ether gave 0.69 g. (41.5%) of pale yellow solid, m.p. 244–249°, $[\alpha]^{22}D$ +57.3° (MeOH). The paper chromatogram showed that it was contaminated with about 5% reserpic acid. The specific activity was 0.37×10^{-4} μc./μM.

Anal. Calcd. for $C_{22}H_{28}N_2O_5 \cdot HCl \cdot 1/2H_2O$ (445.96): C, 59.38; H, 6.78; N, 6.29. Found: C, 59.50; H, 7.07; N, 6.28.

[CONTRIBUTION FROM VARIAN ASSOCIATES, PALO ALTO, AND THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF SAN FRANCISCO, SAN FRANCISCO 17, CALIF.]

Nuclear Magnetic Resonance Spectrum of a Diastereomer of Quercitol (Deoxyinositol). Synthesis of allo-Quercitol and its 6-Chloro, 6-Bromo and 6-Iodo Derivatives[1,2]

BY J. N. SHOOLERY,[3] L. F. JOHNSON,[3] STANLEY FURUTA AND G. E. MCCASLAND[4]

RECEIVED JUNE 6, 1961

A new racemic quercitol (cyclohexanepentol) has been prepared by hydrogenation of *meso*-5,6-anhydro-*allo*-inositol. The new quercitol has been assigned the DL(1234/5) or "allo" configuration on the basis of its nuclear magnetic resonance spectrum and other evidence. Reaction of the same anhydroinositol with concentrated aqueous hydrochloric, hydrobromic and hydriodic acids gave, respectively, a 6-chloro-, 6-bromo-, and 6-iodoquercitol. This is the first known iodoquercitol, and the first chloroquercitol of known configuration. Twenty diastereomeric configurations are predicted for the haloquercitols; the configuration (12346/5) has been assigned to each diastereomer here reported. Pentaacetate derivatives of the quercitol and the haloquercitols were prepared. Configurations are proposed for several other 6-bromoquercitol diastereomers of previously unknown or uncertain configuration. *allo*-Quercitol can also be prepared by hydrogenolysis of a bromoquercitol pentaacetate, m.p. 153°, which Reeves made from *epi*-inositol in 1955.

Nuclear magnetic resonance is one of the most powerful of all techniques for the assignment of diastereomeric configurations, but as yet has been relatively little used for this purpose. This is true in particular in the field of carbohydrates, and one purpose of our present studies is to extend the applicability of n.m.r. to carbohydrate diastereomers. As model compounds, the quercitols[5] (cyclohexanepentols) seemed especially suitable, for two reasons. First, the ten quercitols constitute possibly the largest family of diastereomers in the entire field of organic chemistry *all members of which are known*. Second, unlike ordinary carbohydrates, the quercitols are not subject to complications of structure and conformation resulting from the opening of hemiacetal rings. This initial simplification of the problem is desirable, since carbohydrate n.m.r. spectra[6] are in any event relatively difficult to interpret. The quercitols are also of intrinsic interest because of their natural occurrence and close relationship to that ubiquitous and essential substance, *myo*-inositol.[7]

In a recent publication,[2] the synthesis and n.m.r. characterization of two new quercitol diastereomers (*gala* and *talo*) were described, and a table summarizing all of the ten diastereomers was given. We now wish to report the synthesis of the racemic form of *allo*- or (1234/5)-quercitol (I) and the corresponding 6-chloro, 6-bromo and 6-iodo derivatives (IV-VI), (regarding nomenclature, see our previous article[2]).

The key intermediates used in our present syntheses were *meso*-5,6-anhydro-*allo*-inositol[8] (II) and its 1,2:3,4-diacetone ketal[8] III. The catalytic addition of hydrogen to II gave the quercitol. The addition of hydrogen chloride, bromide or iodide in aqueous solution to III gave, respectively, the chloro, bromo- and iodoquercitol, with simultaneous cleavage of both ketal groups. Because

(1) Presented in part at the Symposium on the Chemistry of Natural Products of the I.U.P.A.C. in August, 1960, at Sydney, Australia. Taken in part from the M.S. Thesis of Stanley Furuta, Graduate Division, University of San Francisco, 1961.

(2) Paper XII on Cyclitol Stereochemistry by G. E. McCasland and co-workers; for preceding paper see: G. E. McCasland, S. Furuta, L. F. Johnson and J. Shoolery, *J. Am. Chem. Soc.*, **83**, 2335 (1961).

(3) Varian Associates.

(4) To whom any requests for reprints should be sent: Address, Department of Chemistry, University of San Francisco, San Francisco 17, Calif.

(5) For an excellent review of previous work on the quercitols, see: (a) S. J. Angyal and L. Anderson in Vol. 14, "Advances in Carbohydrate Chemistry," Academic Press, Inc., New York, N. Y., 1959; also (b) R. L. Lohmer, Jr., in "The Carbohydrates" by W. Pigman (Editor), Academic Press, Inc., New York, N. Y., 1957, pp. 268–296.

(6) For previous work on n.m.r. spectra of carbohydrates and cyclohexane derivatives, see references to work of R. U. Lemieux and others given in Chapter 14 of Pople, Schneider and Bernstein (ref. 11); also see S. Brownstein and R. Miller, *J. Org. Chem.*, **24**, 1886 (1959).

(7) *myo*-Inositol occurs in nearly every plant or animal cell, and is essential for the growth of isolated human cells in tissue culture; see H. Eagle, *J. Biol. Chem.*, **226**, 191 (1957).

(8) S. Angyal and P. Gilham, *J. Chem. Soc.*, 3698 (1957).