sodium hydroxide while 28 g. of dimethyl sulfate was added dropwise. When the *p*H reached 2, sufficient 10% sodium hydroxide was added to bring the *p*H to 10. The mixture was then refluxed for 12 hours. Twenty-five cc. of methyl sulfate was then added and after the *p*H had again reached 2 (1.5 hours), 10% sodium hydroxide was added to return the *p*H to 10. Heating was continued for an additional two hours. (The insoluble sodium salt of the hydroxy indanone reacted slowly with dimethyl sulfate. Thus, this somewhat cumbersome procedure was adopted in order to obtain complete reaction.) The cooled alkaline solution was extracted with ether and the product was distilled (104°, 0.1 mm.) to yield 22 g. (71%) of a liquid which crystallized on standing (m.p. 55–57°).

Anal. Calcd. for $C_{11}H_{12}O_2$: C, 74.97; H, 6.86. Found: C, 75.25; H, 7.15.

7-Hydroxy-3-methylindanone-2-acetic Acid (II).—A mixture consisting of 21.3 g. of 7-methoxy-3-methylindanone, 10.7 g. of dimethylamine hydrochloride, 6.5 g. of paraformaldehyde, 0.5 cc. of concd. hydrochloric acid, and 100 cc. of ethanol was refluxed for six hours, then allowed to stand for two days at room temperature. The solvents were removed *in vacuo* without warming, 100 cc. of water was added, and the mixture was extracted with ether. The combined ether extracts gave 11.4 g. of unreacted 7-methoxy-3-methylindanone. The raffinate was heated at reflux with 10.8 g. of sodium cyanide for five hours, during which time an oil layer separated. The mixture, which stood overnight at room temperature, was extracted with ether to yield 9.0 g. of an oil. (This was presumably crude 7-methoxy-3-methylindanone-2-acetonitrile.) This was heated at reflux for 24 hours with 250 cc. of 10% methanolic sodium hydroxide and the acid isolated as an oil by acidification and extraction with ether. This oil was then heated in a refluxing mixture of 100 cc. of 48% hydrobromic acid and 25 cc. of glacial acetic acid for 18 hours. The solution was cooled, diluted with an equal volume of water and extracted with ether. The product was redissolved in ether and the ether extracted with saturated sodium bicarbonate. Acidification of the bicarbonate solution and extraction with ether gave 3.63 g. of an oil which crystallized immediately when seeded with crystals of isodecarboxyterracinoic acid (II). The m.p. of the crude material (96–105°) was raised by recrystallization from benzene–ethyl acetate to 109.5–110.5°. There was no depression of the m.p. when the sample was mixed with an authentic sample of II. Comparison of the ultraviolet and infrared absorption curves of the two samples confirmed their identity. (The ultraviolet and infrared absorption characteristics of II will be published by Hochstein, *et al.*)

Acknowledgments.—The author gratefully acknowledges the helpful suggestions offered in the course of this work by K. J. Brunings, P. N. Gordon, F. J. Pilgrim, C. R. Stephens and R. B. Woodward. He is indebted to G. B. Hess, J. A. Means and T. J. Toolan for microanalyses and physical measurements. Special thanks are due to F. A. Brastow, A. T. Gilman and R. J. Grabowski for valuable technical assistance.

GROTON, CONNECTICUT

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF TORONTO]

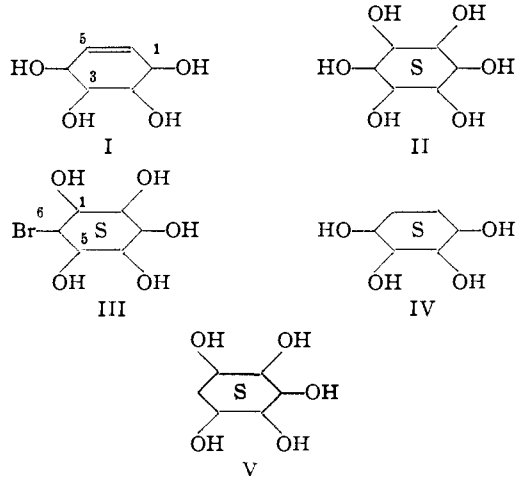
Debromination of Inositol Bromohydrins.* Synthesis of "Conduritol-*B*," *scyllo*Quercitol, and D,L-*vibo*Quercitol[1,2]

BY G. E. MCCASLAND AND E. CLYDE HORSWILL[3]

RECEIVED MARCH 30, 1953

A new cyclohexenetetrol, "conduritol-*B*," has been prepared and shown to have the configuration IX. Dihydroconduritol-*B* is identical with the cyclohexanetetrol m.p. 188° prepared from cyclohexadiene-1,3 by Bedos and Ruyer in 1933. Bedos and Ruyer's other tetrol, m.p. 209°, must then be identical with the dihydro derivative of Kubler's conduritol (conduritol-*A*). Conduritol-*B* tetraacetate is obtained by treating the bromoquercitol pentaacetate of m.p. 240° with zinc–acetic acid. The corresponding free bromoquercitol is 6-bromo*scyllo*quercitol (XI), since it gives *scyllo*quercitol on catalytic debromination. From *myo*inositol another bromoquercitol, m.p. 171°, can be obtained, and it must be 6-bromo*vibo*quercitol (XV), since it gives D,L-*vibo*quercitol on catalytic debromination. The tetraacetate of XV reacts with zinc to give conduritol-*B* identical with the above. The mechanisms of formation and configurations of the known bromoquercitols are discussed.

In 1908 Kubler[4] isolated from the bark of the vine *Marsdenia condurango* the first known cyclohexenetetrol (I), m.p. 143°, which he named *conduritol*.[5] The configuration *meso*-XX for Kubler's conduritol was later established by Dangschat and Fischer.[6] Five other diastereomers of this structure are possible. To avoid ambiguity, the diastereomer XX will be called conduritol-*A* in the remainder of this article.



* Presented before the Organic Division at the Windsor Meeting of the Chemical Institute of Canada, June, 1953.

(1) For related publications on cyclitol chemistry see: (a) G. E. McCasland, THIS JOURNAL, **73**, 2295 (1951); (b) G. E. McCasland and S. Boutsicaris, *ibid.*, **75**, 3845 (1953); (c) H. E. Carter, R. K. Clark, Jr., Betty Lytle and G. E. McCasland, *J. Biol. Chem.*, **175**, 683 (1948); (d) H. E. Carter, C. Belinskey, R. K. Clark, Jr., E. H. Flynn, B. Lytle, G. E. McCasland and Mary Robbins, *ibid.*, **174**, 415 (1948).

(2) From the Ph.D. Thesis of E. Clyde Horswill, 1953.

(3) Fellow of the National Research Council, 1952–1953.

(4) K. Kubler, *Arch. Pharm.*, **246**, 620 (1908).

(5) We use *conduritol* in the generic sense to designate any of the six diastereomers of 5-cyclohexenetetrol-1,2,3,4—also specifically to designate Kubler's conduritol. (*Cf.* usage of *inositol.*) To be fully explicit, Kubler's diastereomer (first one discovered) may be called "conduritol-*A*," and the diastereomer reported by us "conduritol-*B*."

(6) G. Dangschat and H. O. L. Fischer, *Naturwissenschaften*, **27**, 756 (1939).

The double bond in this compound is capable of a great variety of addition reactions. Thus the discovery of conduritol-*A* opened the way to the synthesis of numerous previously inaccessible isomers, analogs or derivatives of inositol. These possibilities were brilliantly utilized by Dangschat and Fischer.[6]

We have been interested for some time in preparing other diastereomers of conduritol which might open up further synthetic possibilities. Unfortunately, attempts to desaturate inositol (II) or other cyclitols by ordinary procedures of dehydration, etc., lead almost invariably to the introduction of three double bonds instead of one (aromatization).

Recently, however, we have found in an obscure observation made by Müller[7] in 1907 the key to the preparation of at least one new conduritol. Müller reported that the pentaacetate (m.p. 240°) of 6-bromoquercitol (III) on treatment with zinc in acetic acid gave a product (A) of m.p. 95° which analyzed for $C_{14}H_{18}O_8$. The exact structure of this product never was reported, and it has apparently received no attention from any subsequent investigator in this field. It seemed to us that Müller's product must have been the tetraacetate of one of the desired cyclohexenetetrols, and evidence now reported confirms this prediction.

Debromination with Zinc: Preparation and Structure of Conduritol-B.—We prepared the bromoquercitol pentaacetate of m.p. 240° from *myo*inositol, and on repetition of Müller's debromination procedure obtained the reported compound *A*, m.p. 92–93°, in 72% yield. Analysis confirmed the formula given above. On deacetylation of *A*, a new unsaturated tetrol, *conduritol-B*, of m.p. 205° was obtained. Analysis agreed with the formula $C_6H_{10}O_4$. The structure was established by hydrogenation to a known, saturated cyclohexanetetrol-1,2,3,4 (IV), with the consumption of 1.03 moles of hydrogen. The saturated tetrol (m.p. 188°) was identical with one of two cyclohexanetetrols prepared from cyclohexadiene-1,3 by Bedos and Ruyer[8] in 1933. A mixed m.p. on the tetrabenzoates (m.p. 260°) was not depressed. Conduritol-*B* must then have the structure (I).

Preparation of Conduritol-*B* from Bromoquercitol Pentaacetate of M.p. 125°.—For the above reaction with zinc the bromoquercitol pentaacetate of m.p. 240° was used; the corresponding free bromoquercitol melts at 224° (dec.).

When *myo*inositol is brominated a second bromoquercitol, m.p. 171°, whose pentaacetate melts at 125°, is also obtained, and in much better yield than the 224° diastereomer. We now find that the pentaacetate of m.p. 125° on treatment with zinc gives conduritol-*B* (tetraacetate) identical with that above, and thus should be a superior starting material.

In order to prove the configurations of conduritol-*B*, the two bromoquercitols, and Bedos and Ruyer's two tetrols, it was necessary to use a different kind of debromination reaction, which will now be described.

(7) H. Müller, (a) *J. Chem. Soc.*, **91**, 1790 (1907); (b) *ibid.*, **101**, 2383 (1912).

(8) P. Bedos and A. Ruyer, *Compt. rend.*, **196**, 625 (1933).

Catalytic Debromination of Cyclitol Bromohydrins.—The work of Müller,[7] Nelson,[9] Wintersteiner,[10] and Carter[11] and their respective coworkers has made available numerous inositol analogs in which one or more hydroxy groups are replaced by bromine (or chlorine), but almost nothing has been known regarding the structures and configurations of these compounds.

By the procedure now described it is possible to replace the bromine in such bromohydrins by hydrogen. In many cases this serves to establish the structure or configuration of the bromohydrin used. The method is also applicable to the preparation of numerous known or new quercitols, cyclohexanetetrols, etc.

The debromination is accomplished by catalytic hydrogenolysis at low pressure, using commercial Raney nickel catalyst, and water as the solvent. Since the hydrobromic acid liberated by the reaction might soon impair the nickel catalyst, Amberlite IR-4B anion-exchange resin is added along with the catalyst to bind the liberated acid.

Formation of *scyllo*Quercitol from "Bromo*scyllo*quercitol."—When the bromoquercitol of m.p. 224° (dec.) was catalytically debrominated by the procedure just mentioned, a 62% yield of *scyllo*quercitol[12a] (XII), m.p. 235°, was obtained.

The identity of our product with authentic *scyllo*quercitol prepared from *scyllo*inosose was confirmed by a mixed m.p., and by X-ray powder spectra on the pentaacetates.

Formation of Racemic *vibo*Quercitol from "Brom*vibo*quercitol."—When the bromoquercitol of m.p. 171° was catalytically debrominated in the same manner, a 48% yield of a quercitol melting at 163° (pentaacetate 113°) was obtained. In all probability this compound is identical with Posternak's[12b,12d] "*d,l*-viburnitol" (XVI) of the same reported m.p.

Configuration and Probable Formation Mechanisms for the Known Bromoquercitols.—Twenty diastereomers of 6-bromoquercitol (III) are theoretically possible. At least three of these are now known (see Table I). The choice of a particular inositol diastereomer as starting material limits the number of bromoquercitol diastereomers obtainable from a particular preparation. It is highly significant that *scyllo*inositol (XXII) and pinitol reportedly give some of the same mono and dibromo products as *myo*inositol itself.

The bromination of an inositol can be carried out either by treating its hexaacetate with hydrogen bromide in acetic acid[7,9] or by treating the free inositol with acetyl bromide.[9–11] The reaction of inositol with aqueous hydrobromic acid is unsatisfactory, probably because pinacolic rearrangement and/or aromatization occurs under these conditions. For the preparation of *mono*bromohydrins we find that a mixture of acetyl bromide and

(9) E. G. Griffin and J. M. Nelson, THIS JOURNAL, **37**, 1552 (1915).

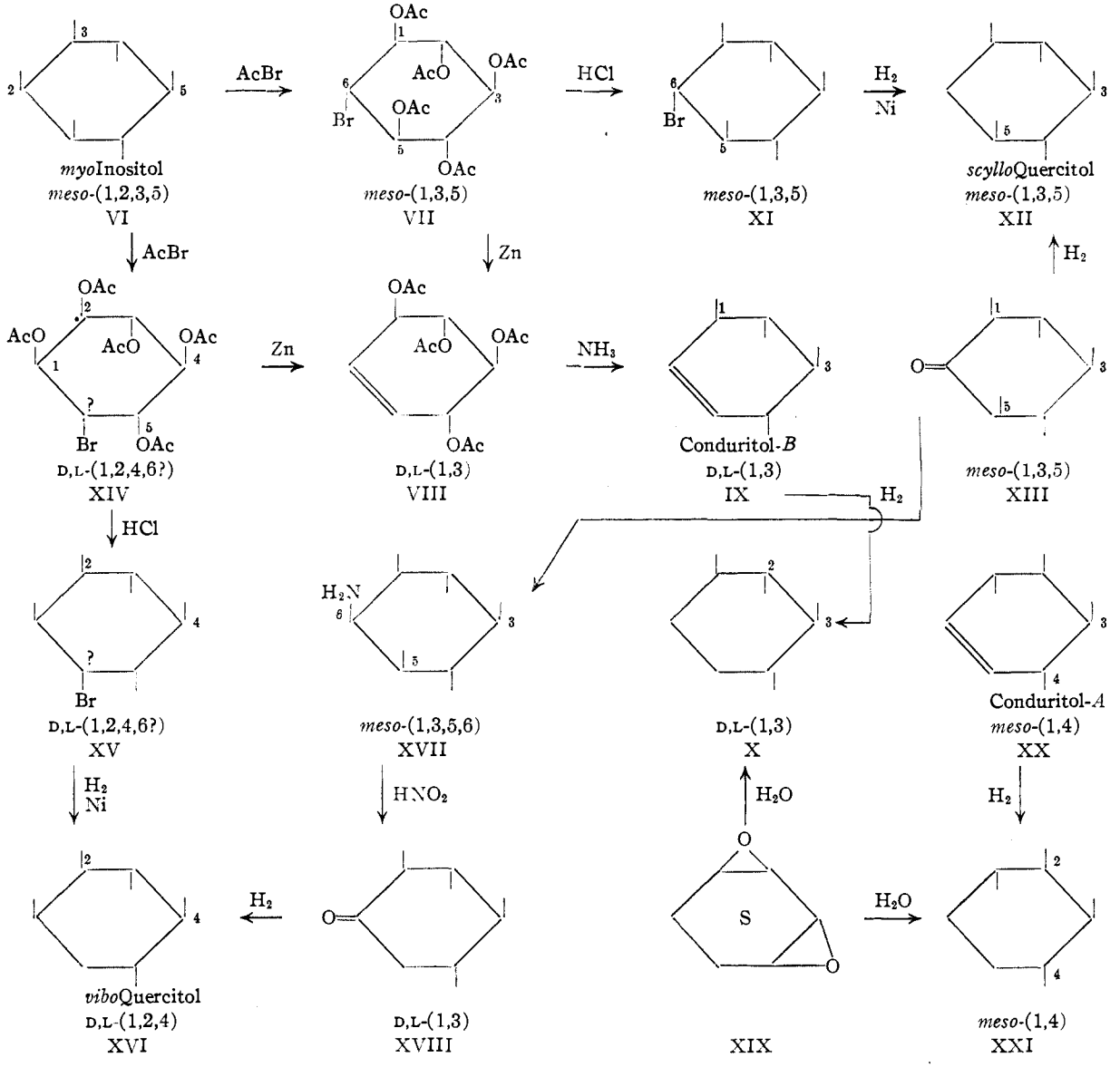
(10) A. E. O. Menzel, M. Moore and O. Wintersteiner, *ibid.*, **71**, 1268 (1949).

(11) E. H. Flynn, Ph.D. Thesis, University of Illinois, 1949 (with Professor H. E. Carter).

(12) Theodore Posternak, (a) *Helv. Chim. Acta*, **24**, 1045 (1941); (b) **33**, 1594 (1950); (c) **33**, 343 (1950); (d) **33**, 1597 (1950); (e) **33**, 350 (1950); (f) **31**, 2242 (1948).

CHART I

DEBROMINATION OF BROMO*scyllo*QUERCITOL-*A* AND OF BROMO*vibo*QUERCITOL-*A*



acetic anhydride is more satisfactory[13] than acetyl bromide alone.

TABLE I

THE KNOWN BROMOQUERCITOLS (1953)

M.p., °C.	Formula	Trivial name	Configuration	Penta-acetate m.p., °C.	Source	Debromination product
171	XXIV or XXV	"Bromo*vibo*quercitol-*A*"	D,L-(1,2,4,6?)	125	*myo*Inositol or *scyllo*inositol, pinitol	D,L-*vibo*Quercitol
175	XXXVIIa or b	"Conduritol-*A* Bromohydrin"	D,L-(1,2,5) or D,L-(1,3,4)	?	Conduritol-*A*	?
223	XXVI	"Bromo*scyllo*quercitol-*A*"	meso-(1,3,5)	240	*myo*Inositol or *scyllo*inositol	*scyllo*Quercitol

The actual reaction seems in any event to be a displacement of one or more acetoxy groups by bromide ion.[14] If the displaced acetoxy group has only *cis* neighboring groups, a simple S_N2 displacement with inversion would be the expected result. However, if either of the two neighboring groups is *trans*, it can participate[15] in the displacement. The result may then be a double inversion, with

(13) Carter and Flynn (ref. 11) using acetyl bromide alone obtained only a 1% yield of bromo*scyllo*quercitol-*A*. Other investigators have not reported yields at all on any of the bromoquercitols or their derivatives—undoubtedly their yields were low.

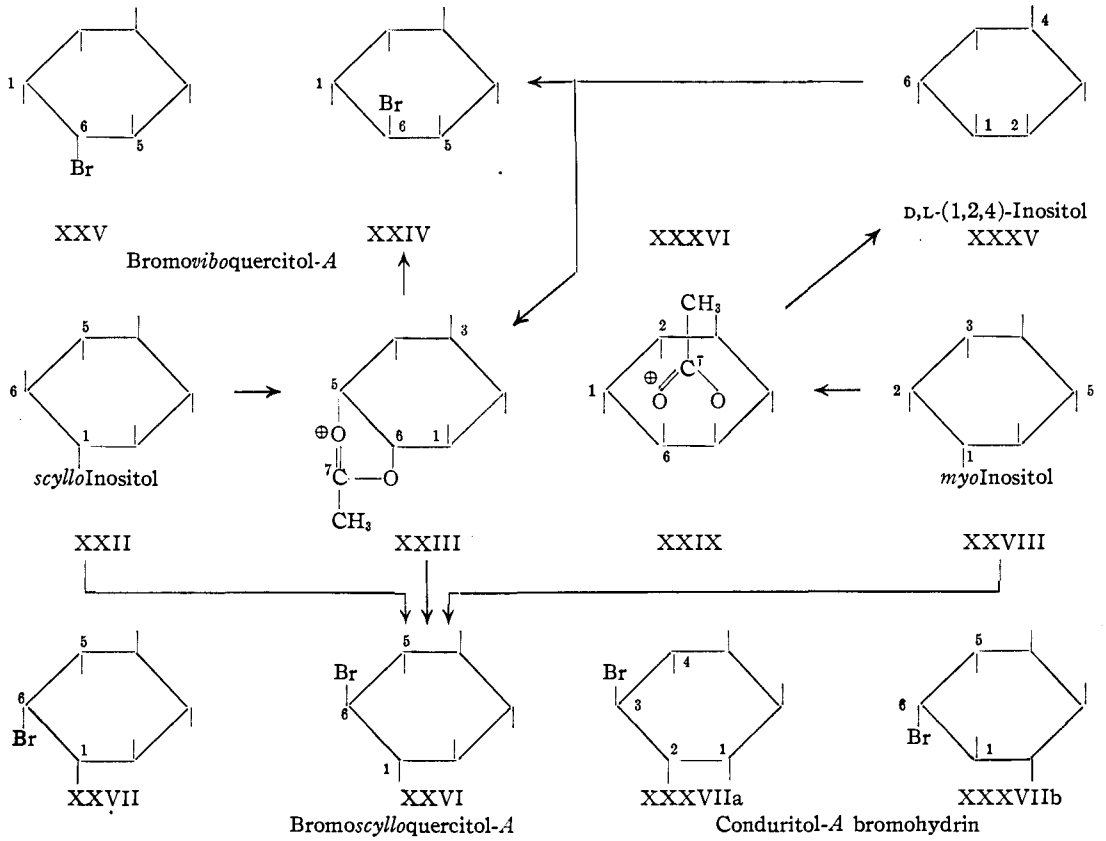
(14) Departure of the acetoxy group in the form of acetic acid is probably facilitated by attachment of a proton from hydrobromic acid to one of the acetoxy oxygen atoms.

(15) The participation of a neighboring acetoxy group in displacement reactions has been discussed in various articles by S. Winstein and co-workers. For example, see THIS JOURNAL, 64, 2796 (1942).

CHART II

REACTION OF ACETYLATED *myo* OR *scyllo*INOSITOL WITH HYDROBROMIC ACID

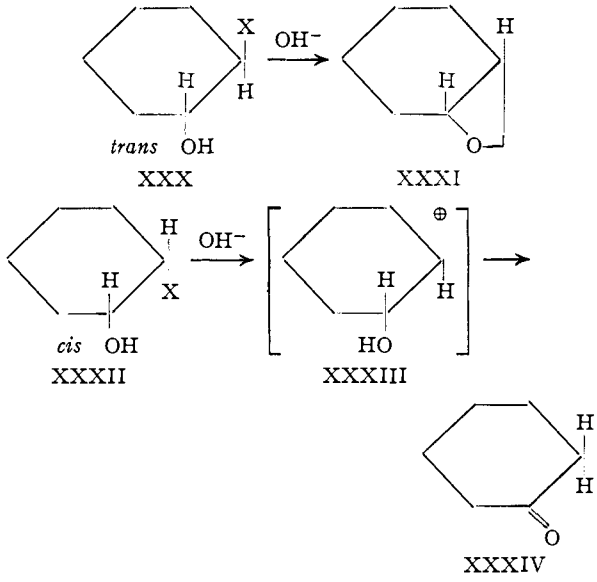
(All hydroxy groups are acetylated. Each formula is independently numbered. The mechanisms indicated seem reasonable but have not been definitely established.)



net retention of configuration. Another effect of the participation may be to cause structural rearrangements, giving two different products (or four, if both neighboring groups are *trans*). By "structural" rearrangement is meant one in which the bromide ion enters a different position than that from which the acetoxy group initially departs; the 6-bromoquercitols can, of course, have only one *structure*. Some of these possible mechanisms are shown in Chart II; however, much further work will be necessary to prove the actual mechanisms.

Configuration of the Bromoquercitol, M.p. 224° ("Bromo*scyllo*quercitol-*A*").—The bromoquercitol pentaacetate of m.p. 240° reported by us is undoubtedly identical with the product of similar m.p. reported by previous workers.[7,9,10] The corresponding free bromoquercitol of m.p. 224° (dec.) is not mentioned in published literature but was prepared by Carter and Flynn in 1948 and described in Flynn's thesis.[11] Since this bromoquercitol on catalytic debromination yields *scyllo*-quercitol, it must be 6-bromo*scyllo*quercitol, for which two diastereomers (XXVI and XXVII) epimeric at position 6 are possible. The epimer of m.p. 224° is hereafter called bromo*scyllo*quercitol-*A*.

The behavior of bromo*scyllo*quercitol-*A* on debromination with sodium hydroxide permits one to choose between the two epimeric configurations. It reacts rapidly at 25° (consuming one mole only of sodium hydroxide) and the reaction mixture gives a negative test for carbonyl products. Bartlett[16] found in analogous studies on the epimeric 2-chlorocyclohexanols that the *trans* epimer XXX reacted rapidly to give the epoxide XXXI, while the *cis* epimer XXXII reacted slowly to give cyclohexanone XXXIV.



(16) P. D. Bartlett, THIS JOURNAL, **57**, 224 (1935).

In the bromoquercitol epimer XXVII the bromine atom would have two *cis* neighboring groups. It is thus reasonable to assign bromo*scyllo*quercitol-*A* the "*trans*" configuration XXVI, since it gives "*trans*" behavior with sodium hydroxide.

Configuration of the Bromoquercitol of M.p. 171° ("Bromo*vibo*quercitol-*A*").—Our bromoquercitol of m.p. 171° is in all probability identical with Müller's product[7] of m.p. 170–175°. No other mention of this diastereomer appears in the literature and the corresponding pentaacetate, m.p. 125°, has never previously been prepared. Müller obtained his product both from *myo*- and from *scyllo*-inositol.

Since this bromoquercitol on catalytic debromination gives D,L-*vibo*quercitol (XVI), and since on debromination with zinc it gives conduritol-*B* (IX), it must be 6-bromo*vibo*quercitol. Two diastereomers epimeric at position 6 (XXIV or XXV) are possible for this compound; the epimer of m.p. 171° is hereafter called bromo*vibo*quercitol-*A*.

The choice between epimers XXIV and XXV is more difficult than that between XXVI and XXVII. Since the bromine atoms in XXIV and XXV each have both *cis* and *trans* neighboring groups, these two epimers might be expected to show similar stereochemical behavior. However, it does seem somewhat more probable that in the formation of conduritol-*B* the eliminated groups at 1 and 6 have a *trans* relationship as in XXIV (*cf.* formation of conduritol-*B* from XXVI).

Configuration of the Bromoquercitol of M.p. 175° ("Conduritol-*A* Bromohydrin").—Kubler's bromoquercitol of reported[4] m.p. 175° prepared by the (presumably *trans*) addition of hypobromous acid to conduritol-*A*, must be either 3-bromodesoxy-D,L-inositol (XXXVIIa) or the bromo*proto*quercitol XXXVIIb, and is thus very probably *not* identical with the bromoquercitols of similar m.p. prepared by Müller and ourselves.

Configurations of Conduritol-*B* and of Bedos and Ruyer's Two Cyclohexanetetrols.—Since it has now been shown that bromo*scyllo*quercitol pentaacetate, has the configuration VII, it is apparent that the acetylated conduritol-*B* prepared from it must have the configuration D,L-VIII. *trans*-Elimination of the bromine atom and *either* neighboring acetoxy group would produce this diastereomer.

Supporting evidence is found in the formation of the same conduritol-*B* tetraacetate from bromo*vibo*quercitol-*A* pentaacetate (XIV). In this case it appears that the *trans* acetoxy group at position 1 (formula XIV) is eliminated in preference to the *cis* group at 5.

In 1933 Bedos and Ruyer[8] prepared two diastereomeric cyclohexanetetrols of m.p. 188° and 210° by hydrolyzing 1,3-cyclohexadiene diepoxide.[17] Since 1,2-epoxycyclohexanes are necessarily *cis*, and on hydrolysis give *trans* diols, it was apparent that these two tetrols could have only the configurations D,L-X and *meso*-XXI. However, it was not known which was which.

A sample of the 188° tetrol prepared from the diepoxide in our laboratory was found to be identical with dihydroconduritol-*B* (D,L-X), as shown by a mixed m.p. on the tetrabenzoates, m.p. 260°.

This means that Bedos and Ruyer's other tetrol of m.p. 210° must have the configuration *meso*-XXI, which would make it identical[17] with the dihydro derivative (reported[6] m.p. 204°) of Kubler's conduritol-*A*.

The preparation of conduritol-*B*, and the proof of configurations for it and for the related bromoquercitols, open up numerous synthetic possibilities, which we are now investigating.

Acknowledgment.—We wish to thank Professor Pierre Bedos of the Université de Toulouse, France, for helpful information and a sample of his cyclohexanetetrol of m.p. 210°. Dr. O. Wintersteiner of the Squibb Institute for Medical Research very kindly sent us a complete set of samples of the bromohydrins prepared from *myo*inositol in his laboratory. We are indebted to Dr. H. E. Carter and Dr. E. H. Flynn for helpful information regarding their studies at the University of Illinois on inositol bromohydrins. We are much obliged to the Research Council of Ontario and to the Advisory Committee on Scientific Research for generous financial support.

Experimental

Note.—Several conflicting systems for the numbering and naming of cyclitols are at present in use. The significance of the names in this article will no doubt be apparent on consulting the corresponding perspective formulas in Charts I and II. The names "*scyllo*quercitol" and "*vibo*quercitol" were suggested by Dr. S. J. Angyal of the University of Sydney, Australia (see *J. Chem. Soc.*, 686 (1952)).

All melting points (corrected) taken on Köfler microblock, except mixed m.p.'s (capillary). Microanalyses by Micro-Tech Laboratories, Skokie, Illinois.

***meso*-(1,3,5)-*A*-6-Bromoquercitol Pentaacetate ("Bromo*scyllo*quercitol-*A* Pentaacetate"), VII. (A).**—The procedure of Griffin and Nelson[9] was modified as follows. A mixture of 2.5 g. of pulverized anhydrous *myo*inositol, 2.2 ml. of acetyl bromide and 6.5 ml. of redistilled acetic anhydride was heated at 120° for six hours in a "semi-micro" sealed tube.[18] After cooling, the entire tube contents were transferred to a flask containing 20 ml. of absolute ethanol, with the aid of 5 ml. of additional ethanol. The combined mixture after 24 hours at 25° was filtered, washed with absolute ethanol, and dried, giving 0.77 g. of a greyish-white crystalline powder. On recrystallization from absolute ethanol, 0.67 g. (11%) of colorless, matted, microscopic needles, m.p. 241–241.5°, were obtained.

Mixed m.p.'s with a sample prepared by us using the original Griffin–Nelson procedure,[9] and with a sample prepared by Wintersteiner,[10] *et al.*, showed no depression.

Preliminary attempts to use a larger scale procedure (10–20 g. of inositol per tube) have given yields only about half as large.

(B).—Attempted catalysis of the reaction by adding small amounts of zinc bromide or ferric bromide decreased the yield to 0.40–0.54 g.

(C).—Dry commercial hydrogen bromide gas was passed continuously through a boiling solution of *myo*inositol hexaacetate (2.16 g.) in 50 ml. of glacial acetic acid under reflux for 24 hours. The yellow solution was then vacuum distilled down to an orange sirup (2.42 g.), which was dis-

(17) Just before submitting this manuscript we received a publication by T. Posternak and H. Friedli reporting that they have taken a mixed m.p. on samples of cyclohexanetetrol, m.p. 210°, from conduritol and from cyclohexadiene, showing conclusively that the two products are identical (see *Helv. Chim. Acta*, **36**, 251 (1953)).

(18) The reaction is best carried out in a Pyrex semi-micro bomb tube (15 × 150 mm.). An electric bomb furnace, *e.g.*, Sargent 36460, is convenient. The yield was smaller using a Corning 8560 macro tube (25 × 600 mm.), possibly because of hindered dissipation of heat from the initial exothermic reaction.

solved in 50 ml. of boiling absolute ethanol. On cooling, 0.12 g. (5%) of colorless needles, m.p. 240–240.5°, was obtained. Longer reaction times increased the yield only slightly; shorter times caused contamination of the product with starting material.

meso-(1,3,5)-*A*-6-Bromoquercitol ("Bromo*scyllo*quercitol-*A*"), XI.—The pentaacetate of m.p. 240° (2.27 g.) was boiled with 45 ml. of a 1.0 *N* solution of hydrogen chloride in 48% ethanol for six hours, followed by vacuum distillation to near dryness. Absolute ethanol was added and distillation to dryness repeated several times. The final residue consisted of 1.35 g. of colorless powdery crystals, m.p. 217–225° dec. Recrystallization from methanol (120 ml.) plus benzene (240 ml.) gave 1.01 g. (83%) of colorless flakes, m.p. 222–223.5° dec. A sample recrystallized once more for analysis melted at 223–224° dec. The compound melts at 225–227.5° if the stage is preheated to 215°.

Anal. Calcd. for $C_6H_{11}BrO_5$: C, 29.65; H, 4.56; Br, 32.88. Found: C, 30.04; H, 5.03; Br, 33.12.

D,L-(1,2,4,6?)-R-6-Bromoquercitol Pentaacetate ("Bromo*vibo*quercitol-*A* Pentaacetate"), XIV. (A).—When the ethanolic mother liquors from bromo*scyllo*quercitol pentaacetate (method A, above) were allowed to stand at 25° for 1 to 12 weeks, crystals of crude (deacetylated) bromo*vibo*quercitol-*A* (m.p. 155–170°) gradually[19] precipitated. From 60 g. of inositol there were obtained 17.1 g. (22%) of crystals after one week, and a total of 47.1 g. (59%) after three months.

The crude bromoquercitol (12.2 g.) was mixed with 95 ml. of acetic anhydride and 5.0 ml. of sulfuric acid. After 24 hours at 25°, the solution was poured onto 0.5 kg. of crushed ice. The filtered, washed and dried product was a white powder of wide melting range, weight 17.4 g. It was recrystallized from absolute ethanol (300 ml.). After 24 hours a first crop (0.56 g.), consisting apparently of acetylated bromo*scyllo*quercitol, m.p. 239–239.5°, was removed. After two days more, a second crop of 14.2 g., m.p. 107.5–109°, was obtained. The second crop was again recrystallized from absolute ethanol, giving 12.7 g. of acetylated bromo*vibo*quercitol, colorless prisms, m.p. 106–108°. The product at this stage is sufficiently pure for the zinc–acetic acid reaction (see below).

For further purification the product can be chromatographed.[20] A solution of 0.50 g. in 3.0 ml. of dry benzene was passed through a 12 × 190 mm. column of aluminum oxide (acetic acid washed, and dried at 150°/48 hr.). The column was eluted with 100 ml. of dry benzene. The first 10-ml. fraction gave on evaporation a colorless sirup which soon crystallized (0.37 g.). After three recrystallizations from absolute ethanol 0.25 g. of colorless microscopic sheaves of elongated prisms was obtained, m.p. 124–125° (after partial liquefaction at 108° and resolidification). From the next 20-ml. fraction of eluate, 0.08 g. more crystals was obtained.

Anal. Calcd. for $C_{16}H_{21}BrO_{10}$: C, 42.40; H, 4.67; Br, 17.63. Found: C, 42.69; H, 4.90; Br, 18.50.

(B).—Vacuum distillation to near-dryness of the ethanolic mother liquors from bromo*scyllo*quercitol prepared by method (C) above gave 1.96 g. of discolored sirupy crystals. These were recrystallized from ethanol (2.2 ml.) giving 0.86 g. (38%) of colorless prisms, m.p. 99.5–104.5°. After another recrystallization 0.60 g. of m.p. 103.5–106.5° was obtained.

D,L-(1,2,4,6?)-R-6-Bromoquercitol ("Bromo*vibo*quercitol-*A*"), XV.—The pentaacetate (3.13 g.) was hydrolyzed by the same method used for bromo*scyllo*quercitol. After reprecipitation from methanol by adding benzene, 1.49 g. (88%) of colorless crystals, m.p. 167–170° (dec. 180°) was obtained. A sample recrystallized once more for analysis melted at 169.5–171°. The product is presumably identical with Müllers bromoquercitol of reported m.p. 170–175°.

(19) The gradual separation of bromo*vibo*quercitol-*A* from the ethanol is perhaps due to the slow alcoholysis of the acetate ester groups, catalyzed by the hydrobromic acid which is present. The partially deacetylated bromohydrins reported by Griffin and Nelson may have a similar explanation. However, it is also possible that an intermediate such as XXIII is attacked by acetic acid at position 7 (instead of 5 or 6) to give an orthoacetate structure which opens up to give a diol *mono*acetate structure (see Winstein, *et al.*, THIS JOURNAL, **64**, 2796 (1942)).

(20) Wintersteiner, *et al.*, ref. 10, used a similar chromatographic technique.

D,L-(1,3)-R-5-Cyclohexenetetrol-1,2,3,3 Tetraacetate ("Conduritol-*B* Tetraacetate"), VIII. (A). (From Bromo*vibo*quercitol-*A* Pentaacetate).—A 4.53-g. portion of bromo*vibo*quercitol pentaacetate (m.p. 106–108°) in 50 ml. of glacial acetic acid was stirred with 4.5 g. of powdered metallic zinc under anhydrous conditions for 24 hours on the steam-bath.

The presence of bromo*scyllo*quercitol-*A* pentaacetate as impurity in the starting material does no harm, since it yields the same product with zinc–acetic acid.

After cooling, the reaction mixture was filtered and the inorganic residue washed with two 5-ml. portions of ether. The combined filtrates were vacuum distilled down to a pale brown sirup. The sirup when rubbed with 10 ml. of warm water gave crystals which when collected, washed and dried, weighed 2.85 g. (91%), m.p. 80–85°. After recrystallization from ethanol–water 2.31 g. of m.p. 87–92° was obtained. After a second recrystallization the weight was 2.09 g. (67%) and the m.p. 91–92.5°.

Anal. Calcd. for $C_{14}H_{18}O_8$: C, 53.50; H, 5.77. Found: C, 53.85; H, 5.80.

(B). (From Bromo*scyllo*quercitol-*A* Pentaacetate).—When bromo*scyllo*quercitol-*A* pentaacetate (1.81 g.) in 20 ml. of acetic acid was treated with 1.81 g. of powdered zinc at 70° for 17 hours, 1.17 g. of colorless crystals, m.p. 80–87°, was obtained. The product was dissolved in boiling ethanol–water, and the crystals which separated on cooling were collected by centrifugal filtration in a Skau tube, weight 0.90 g. (72%). The crystals melted at 92–93° and a mixed m.p. with the product prepared from bromo*vibo*quercitol showed no depression.

D,L-(1,3)-R-5-Cyclohexenetetrol-1,2,3,4 ("Conduritol-*B*"), IX.—The tetraacetate (0.90 g.) was dissolved in 50 ml. of a saturated solution of dry ammonia in absolute methanol. After 24 hours at 25° the solution was filtered, and vacuum distilled to dryness. Acetamide was removed from the residue by vacuum-sublimation at 70° (8 mm.). The colorless residue weighed 0.40 g. (95%) and melted at 203–204°. The residue was dissolved in 15 ml. of boiling methanol, the solution filtered and 30 ml. of warm benzene added. The crystals obtained (small prisms) weighed 0.30 g. (72%) and melted at 204.5–205°.

A sample recrystallized once more for analysis showed no change in m.p. An aqueous solution of the product gave a positive bromine water test for unsaturation.

Anal. Calcd. for $C_6H_{10}O_4$: C, 49.31; H, 6.89. Found: C, 49.60; H, 6.89.

D,L-(1,3)-R-Cyclohexanetetrol-1,2,3,4 ("Dihydroconduritol-*B*"), X.—Conduritol-*B* (0.146 g.) when hydrogenated at 1 atm. (25°) in 15 ml. of methanol, using Adams catalyst, consumed 1.03 moles of hydrogen per mole of compound. The filtered mixture on vacuum distillation gave 0.110 g. (74%) of colorless crystals, m.p. 179–186.5°.

A sample (107 mg.) recrystallized from methanol gave 70 mg. (49%) of crystals melting at 187–188°.

This cyclohexanetetrol is identical with the tetrol prepared by Bedos and Ruyer[3] in 1933 from cyclohexadiene-1,3 *via* the diepoxide. Bedos and Ruyer's preparation was repeated by us and the identity of the two products shown by a mixed m.p. on the tetrabenzoates.

D,L-(1,3)-R-Cyclohexanetetrol-1,2,3,4 Tetrabenzoate ("Dihydroconduritol-*B* Tetrabenzoate").—Dihydroconduritol-*B* when heated with benzoyl chloride in pyridine for one hour under reflux gave a crude tetrabenzoate, m.p. 190–235°. After two recrystallizations from absolute ethanol, short microscopic needles of m.p. 259–260° were obtained.

Benzoylation in a similar manner of the cyclohexanetetrol (m.p. 188°) prepared by the method of Bedos and Ruyer gives an identical product.

meso-(1,3,5)-A-Quercitol ("*scyllo*Quercitol," XII), from Bromo*scyllo*quercitol-*A*.—To 0.90 g. of the bromoquercitol (m.p. 224° dec.) in 50 ml. of water was added 1.5 g. (dry weight) of Amberlite IR-4B anion-exchange resin and 10 g. (wet) of commercial Raney nickel catalyst (washed until neutral). On hydrogenation at three atm., 1.07 moles of hydrogen per mole of compound was consumed. The filtered mixture was vacuum distilled, giving a residue of 0.61 g. of colorless crystals, m.p. 204–218°. The product (0.50 g.) was recrystallized from 2-methoxyethanol; the crystals when washed with butanone and dried weighed 0.31 g. (62%), m.p. 232–234.5°. A second crystallization raised the melting point to 233.5–234.5°.

The product was identical with *scyllo*quercitol.[12a] Admixture of authentic *scyllo*quercitol prepared by us from *scyllo*inosose did not depress the m.p. A portion was converted to the pentaacetate, m.p. 193–194°. Identity of the two acetates was demonstrated by X-ray powder pictures.

D,L-(1,2,4)-R-Quercitol ("D,L-*vibo*Quercitol," **XVI**) **from Bromo*vibo*quercitol-*A*.**—Bromo*vibo*quercitol (m.p. 171°) was catalytically debrominated as above. Traces of nickel ion were removed from the hydrogenation filtrate by the use of Amberlite IR-120 (H^+) resin until a negative dimethylglyoxime test was obtained. The resulting acidic solution was neutralized by treatment with IR-4B resin, filtered, and the filtrate vacuum-distilled to a small volume.

On addition of 25 ml. of absolute ethanol, crystals separated. The solvent was removed by vacuum-distillation, giving 0.51 g. (77%) of colorless crystals, m.p. 154–158°. Recrystallization from 95% ethanol gave 0.32 g. (48%) of crystals, m.p. 161–163°.

The product appears to be identical with the D,L-*vibo*-quercitol of Posternak,[12b] reported m.p. 161–163°.

A portion acetylated with acetic anhydride gave D,L-*vibo*-quercitol pentaacetate of m.p. 112–113°, reported[12b] m.p. 113–114°.

Reaction of Bromo*scyllo*quercitol-*A* with Sodium Hydroxide.—A 0.4861-g. portion of bromo*scyllo*quercitol (m.p. 224° dec.) was dissolved in sufficient 0.1611 *N* sodium hydroxide to make 50.00 ml. Aliquots of 5.00 ml. were withdrawn at intervals for titration with 0.1047 *N* hydrochloric acid (phenolphthalein), with the results shown:

Time, min.	NaOH consumed, moles/mole	Time, min.	NaOH consumed, moles/mole
0	0	73	0.95
8	.49	126	0.99
28	.74	268	1.00
45	.88	1455	1.02

The last five neutralized samples were combined, and deionized by successive treatment with Amberlite IRA-400 and IR-120 (H^+) resins. The filtered solution on vacuum distillation left a colorless oil as residue. A portion of the oil was dissolved in ethanol and gave a negative test for carbonyl groups with dinitrophenylhydrazine–phosphoric acid reagent.[21]

The remainder of the oil crystallized after standing for three weeks with ethanol, m.p. 146–151°. The crystals gave a negative Beilstein halogen test, and are rather soluble in water. The exact nature of this compound has not yet been determined; presumably it is an epoxycyclohexanetetrol.[22]

(21) C. D. Johnson, THIS JOURNAL, **73**, 5888 (1951).

(22) Cyclohexene oxide is quite stable with dilute aqueous alkali (partly because of low solubility), but the polyhydroxyepoxide presumably formed here would perhaps be more reactive. It might be hydrolyzed to an inositol before isolation is possible.

TORONTO, CANADA

[CONTRIBUTION FROM THE DEPARTMENT OF PHYSIOLOGICAL CHEMISTRY, SCHOOL OF MEDICINE, UNIVERSITY OF CALIFORNIA]

Some Carboxypeptidase–Substrate Relationships[1]

BY EDWARD RONWIN[2]

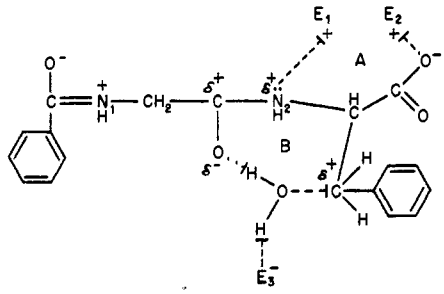
RECEIVED APRIL 11, 1953

In accordance with the dipositive-bond theory, N-*p*-toluenesulfonyl-DL-phenylalanine and N-chloroacetyl-DL-aspartic acid were found to be resistant to the enzyme. Several representatives of N-chloroacetylated, N-trichloroacetylated and N-hippurylated amino acids have been subjected to the action of the enzyme for the first time. There are indications of a substrate inhibition in the case of chloroacetyl-DL-tyrosine. At first approximation, the trichloroacetate ion is a strong inhibitor. Contrary to the notion that the greater the acid strength of the acyl moiety, the greater the rate of hydrolysis; the trichloroacetyl derivatives have been found to be much poorer substrates than the corresponding chloroacetyl derivatives of amino acids. It was unexpectedly observed that hippurylleucine is a better substrate than hippuryltyrosine. The results, in general, were as anticipated and demonstrate that hippuryl derivatives of amino acids are, thus far, the most potent synthetic carboxypeptidase substrates.

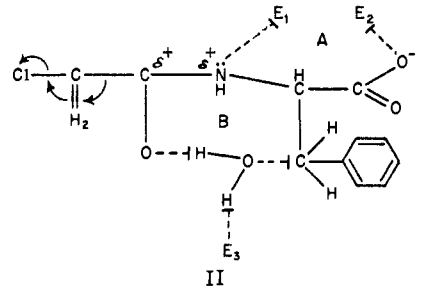
Introduction

The recently suggested dipositive-bond theory[3] serves as the stimulating principle of these studies. The basic tenets of this theory are two in number: (1) the susceptible bond is ruptured or considerably weakened as a result of the creation of a dipositive charge situation at the hydrolyzable link which is in turn caused by the nature of the bonding of the substrate to the enzyme and (2) the formation of a fruitful ES complex, with the ensuant dipositive situation, involves the simultaneous creation of two rings in the union of enzyme and substrate. This is illustrated by I for N-substituted dipeptide substrates and by II for the N-acylated amino acid substrates of the enzyme (beef pancreatic carboxypeptidase) that is being considered here.

According to the dipositive-bond theory, the contribution to substrate susceptibility of the group attached to the nitrogen atom of the antipode am-



I[4]



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(1) Part of this work was performed during the tenure of a Life Insurance Medical Research Fellowship. The manuscript was originally received June 11, 1952.

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(3) The theory and its application to several enzymes is discussed in detail in the Ph.D. thesis of the author. Also, *Enzymologia*, in press.

(4) The symbol, -----|, represents any bond between oppositely charged groups on the enzyme and substrate. It is always written with the head adjacent to the positive area.