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BROMINE OXIDATION OF INOSITOLS FOR PREPARATION OF INOSOSE PHENYLHYDRAZONES AND PHENYLOSAZONES

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ABSTRACT

The application of bromine oxidation of inositols to give inososes, followed by conversion of the latter into phenylhydrazones or phenylosazones, is described. The diketone from *myo*-inositol gives a phenylosazone in 22–30% yield, and L-inositol gives a monoketone phenylhydrazone (12% yield) and a diketone phenylosazone (28% yield), the corresponding enantiomorphs were obtained in 8% and 29% yield, respectively The diketone from quebrachitol yields a new phenylosazone (29% yield) and no monoketone was isolated Pinitol gave a new diketone phenylosazone (10–15% yield) which showed rapid mutarotation in 1 1 (v/v) ethanol–*p*-dioxane In addition, a new phenylosazone has been obtained from DL-*epi*-inosose-2 phenylhydrazone (+)-*proto*-Quercitol (from acorns) has been converted into a phenylosazone (20% yield) *myo*-Inositol has been converted by bromine oxidation into DL-*xylo*-pentahydroxy-2-cyclohexen-1-one in low yield

INTRODUCTION

Methods for the preparation of "osones" and other dicarbonyl derivatives of sugars have received extensive study[1,2] For dicarbonyl derivatives of inositols, oxidation by *Acetobacter suboxydans* has been used most[3,4], alternatively, oxidation of inososes or their phenylhydrazones in the presence of phenylhydrazine and acetic acid has been employed[5]

The application of sodium hypobromite for oxidation of cyclitols has been reported[6–8]. With sugars, bromine in buffered solution at low temperature oxidizes aldoses quantitatively to their corresponding lactones[9], whereas ketoses are essentially unaffected under these conditions Glycosides are known to undergo oxidation under more vigorous conditions[10].

Except for a general statement by Posternak[11], in which he claims that, on hypobromite oxidation of inositols, yields of inososes are not satisfactory, we are

*Part III Methods in Inositol Chemistry For Part II, see *Carbohyd Res*, 6 (1968) 489

unaware of any published report on oxidation of inositols with bromine to give monoketoinositols (inososes), although D-quinic acid (D-1,3-dideoxy-*epi*-inositol-2-carboxylic acid) gives[12] a monoketo derivative in 60% yield, and oxidation of 1,2,3-*cis*-cyclohexanetriol with hypobromite gives[13] a monoketone in a yield of about 2%

A re-examination of the usefulness of bromine for oxidation of cyclitols is the subject of this paper.

In the previous applications of sodium hypobromite as an oxidant in the cyclitol field, preparation of cyclitol diketones was described, and these products were isolated as phenylosazones; for example, on a 1-gram scale, *myo*-inositol was oxidized with bromine in the presence of sodium carbonate buffer, and addition of phenylhydrazine gave[8] DL-*myo*-inosose-1 phenylosazone* in a yield of about 7%. Similarly, phenylosazones have been obtained from oxidation of (+)-1-deoxy-*muco*-inositol [(+)-*proto*-quercitol][6] with aqueous bromine, and from oxidation of (−)-1-deoxy-*myo*-inositol [(−)-viburnitol] with sodium hypobromite[7], however, the yields were not stated

RESULTS AND DISCUSSION

Initially, the oxidation of *myo*-inositol and of L-inositol by bromine in sodium carbonate and in sodium acetate was examined Preliminary evidence (t l c) indicated that both mono- and di-ketones were formed in significant proportions in the oxidation mixtures The yield of each product appeared to be greater in the oxidations employing the acetate buffer Consequently, acetate-buffered oxidations were then studied exclusively

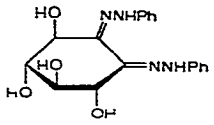
The ice-cold solution of the cyclitol was oxidized with a *ca* 35% molar excess of bromine (calculated for mono-oxidation) in the presence of sufficient sodium acetate to buffer all of the hydrobromic acid that might be formed The oxidation products were isolated by conventional methods, either as phenylhydrazones or as phenylosazones For the latter, the concentration of the oxidized cyclitol was adjusted by dilution with water to 3–6% (pH 5–6) to obtain the maximal yield of the osazone Over-oxidation was observed if the cyclitol–bromine mixture was kept for 48 h or longer; the phenylosazones from over-oxidized mixtures (96 h) were found to contain considerable proportions (3–5%) of a red pigment that was difficult to remove

Addition of phenylhydrazine to the oxidation product of *myo*-inositol gave DL-*myo*-inosose-1 phenylosazone (**1** and **2**) only, in 22–30% yield; none of the monoketone was isolated Starting with L-inositol, however, D-*myo*-inosose-1 phenylhydrazone (**3**) was obtained in 12% yield, and, alternatively, D-*myo*-inosose-1 phenylosazone (**2**) was isolated in 28% yield D-Inositol gave the corresponding enantiomorphs **4** and **1**, in 8 and 29% yields, respectively.

T l c showed evidence of both the mono- and the di-keto products from quebrachitol [1-*O*-methyl-*levo*-inositol] and pinitol [5-*O*-methyl-*dextro*-inositol],

*The nomenclature of this and other inositol derivatives used in this paper is based on the Fletcher–Anderson–Lardy system of H G Fletcher, Jr , L Anderson, and H A Lardy, *J Org Chem* , 12 (1951) 1238, see also ref 14

but only the diketo derivatives of each were actually isolated (as phenylosazones, compounds **5** and **6**, respectively)



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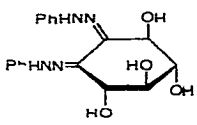

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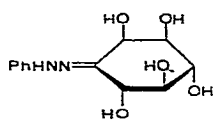

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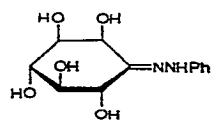

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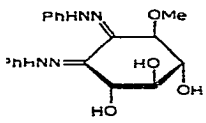

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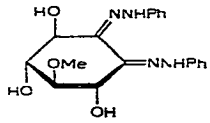

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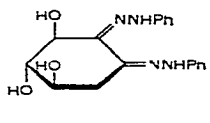

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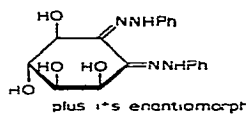

plus its enantiomorph

8

Efforts to obtain either monoketo derivative as a phenylhydrazone were unsuccessful, mainly because of their high solubility. Although the yield of phenylosazone **5** was 29%, the yield of **6** was only 10–15%

The crude phenylosazone **6** contained a considerable proportion of an unknown, yellow component having a higher R_F value (see Experimental Section), in addition to several red-orange components, the mixture also contained a small proportion (2%) of L-*myo*-inosose-1 phenylosazone. this can arise from the parent compound by demethylation to D-inositol, followed by oxidation of the latter to mono- and di-keto derivatives

The mutarotation of phenylosazone **6**, exhibiting a rapid change from $[\alpha]_D$ -507 to 0.00°, followed by a slow change to $+130°$, indicates occurrence of at least two reactions The mutarotation of **6** must be associated with the presence of a remote 5-methoxyl group, as phenylosazone **5**, having a 3-methoxyl group, shows no mutarotation*

Under similar conditions of reaction, quebrachitol proved to be more stable toward bromine oxidation, and the crude phenylosazone **5** isolated contained only a red component; practically no demethylation was observed

Compound **5** and **6** have not been reported previously They were identical in all physical properties with the phenylosazones which we have prepared in two steps by (*1*) catalytic oxidation of quebrachitol and pinitol to the corresponding inososes as described by Post and Anderson[14], and (*2*) further conversion into their osazones Column chromatography of the crude phenylosazone **6** obtained by catalytic oxidation of pinitol (through pinitol inosose followed by treatment with phenylhydrazine) showed the presence of the same yellow and red impurities as were observed in the product from bromine oxidation On the other hand, quebrachitol was found to be more stable toward oxidation, and the phenylosazone **5** isolated contained less of the decomposition products than phenylosazone **6**

By bromine oxidation in acetate buffer, (+)-*proto*-quercitol gave the diketone

*In general the specific rotations of inosose phenylosazones in 1 1 (v/v) ethanol–*p*-dioxane are somewhat higher than those in 1·1 (v/v) ethanol–pyridine

phenylosazone (7) in approximately 20% yield (based on the average concentration of (+)-*proto*-quercitol present in dried, ground acorns)

In the presence of sodium pivalate or propionate and their corresponding acids (instead of sodium acetate–acetic acid) as buffers in oxidations with phenylhydrazine of the inososes to their phenylosazones, the products initially isolated were less colored, and were therefore considered to be of higher quality, than those obtained in the acetate buffer. Substitution of hydrochloric, sulfuric, or formic acid for the buffer gave no phenylosazone

Excess bromine may conveniently be removed by addition of acrylamide, acrylonitrile, diethylamine, methyl methacrylate, or sodium hydrogen sulfite, or by bubbling of ethylene–air mixture, sodium hydrosulfite or sodium thiosulfate interfere in the redox cycle that is apparently necessary for the formation of the phenylosazone, and therefore cannot be used The red pigment present in crude inosose phenylosazones can conveniently be removed by treatment of the crude product with a warm, aqueous ethanolic solution of sodium hydrosulfite ($Na_2S_2O_4$)

It is recommended that final solutions containing D- or[14] L-*myo*-inosose-1 intended for conversion into the corresponding phenylosazones be diluted with methanol (or ethanol) up to 30%, prior to treatment with phenylhydrazine, in order to keep the sparingly soluble D- or L-*myo*-inosose-1 phenylhydrazones in solution, otherwise, the inosose phenylosazone obtained may contain a considerable proportion of the phenylhydrazone. In cases where a mixture of inosose phenylhydrazone and phenylosazone is suspected, the quick, resin test[15] reveals the presence of the reducing component (inosose), if it is present, the mixture is dissolved in warm methanol and re-treated with phenylhydrazine in aqueous acetic acid; the conversion of a solid inosose phenylhydrazone into the phenylosazone under the conditions described is incomplete

It may be noted, however, that inososes or their phenylhydrazones may differ in their rates of conversion into the corresponding phenylosazones under the conditions described For example[16], DL-*epi*-inosose-2 or[17] L-*epi*-inosose-2 or their phenylhydrazones are more difficult to convert into the corresponding phenylosazones under the conditions given; this indicates thermodynamic stability of L-*epi*-, or D-*epi*-, or DL-*epi*-inosose-2 phenylhydrazones, as compared to the readily convertible *myo*-inosose-2 or D- or L-*myo*-inosose-1 phenylhydrazones The Fiesers[18] attributed the slow formation of DL-*epi*-inosose-2 phenylosazone (8) from its phenylhydrazone to its having a chair conformation, which restricts the plane of the C=N bond from becoming parallel to a HCOH group and thus retards the Amadori hydrogen shift, the possible mechanisms for the formation of the phenylosazones from sugars[19,20] or other compounds[21], including the Amadori-type rearrangement, have recently been discussed The difficulty in preparation of DL-*epi*-inosose-2 phenylosazone (8), under the conditions described, could also be ascribed to the moderate solubility of DL-*epi*-inosose-2 phenylhydrazone; this compound requires a considerable proportion of methanol (or other suitable solvent) to keep it in solution. A small quantity of the

previously unreported DL-*epi*-inosose-2 phenylosazone (**8**) has now been prepared by a laborious procedure

The action of bromine on inositols is stereospecific and can be compared with catalytic or bacterial oxidants which, as is known, show a tendency to attack axial hydroxyl groups

Oxidation of *myo*-inositol with potassium hypobromite for 36 h, followed by neutralization with potassium carbonate and cooling, gave, in low yield, the crystalline potassium salt of DL-*xylo*-pentahydroxy-2-cyclohexen-1-one, an enolic form of a diketoinositol prepared by the oxidation of *myo*-inositol with nitric acid[22]. It is interesting that both oxidants (bromine and nitric acid) produce the same diketoinositol intermediate

EXPERIMENTAL

Materials — *myo*-Inositol was a purified, commercial* preparation (Corn Products Refining Co., Argo, Illinois) Crude quebrachitol* (Plant Division, U S Rubber Co., Rockefeller Center, New York, N Y) was purified by recrystallization (carbon) from aqueous ethanol containing 10% of acetic acid (solvent *A*), m p 191–193°, $[\alpha]_D^{22}$ −80 5° (*c* 2 37, water) Crude pinitol* (Western Pine Association Research Laboratory, Portland, Oregon) was purified by recrystallization from solvent *A*, m p 185–186°, $[\alpha]_D^{22}$ +66° (*c* 2 5, water) L-Inositol was prepared by demethylation of purified quebrachitol A mixture of quebrachitol (25 g) and 47% hydriodic acid (50 ml) was refluxed for 30 min, the warm solution was added portionwise to warm absolute ethanol (400 ml) with stirring, yield of crude L-inositol, 20–22 g; after recrystallization from warm aqueous ethanol, it had m p 243–244°, $[\alpha]_D^{22}$ −64 9° (*c* 2 0, water) D-Inositol was prepared by demethylation of pinitol by the same procedure, after recrystallization from warm aqueous ethanol, it had m p 246–248° $[\alpha]_D^{22}$ +65.1° (*c* 2 2, water)

I r spectra were recorded with a Perkin–Elmer 257 grating spectrophotometer*, the absorption bands (see Table I), particularly those found for N-H (bending) or Ph-N groups of inosose phenylhydrazones or phenylosazones, were in good agreement with those found for other mono-, bis-, and tris-(phenylhydrazones)[23] U v and visible spectra were recorded with a Beckman DK-2 spectrophotometer*, all inosose phenylosazones showed absorption bands at 254–259, 308–321, and 389–399 nm, as does D-*myo*-inosose-1 phenylosazone[24] However, many inosose phenylosazones show, in addition to the three bands, a broad band or a shoulder in the region at 227–230 nm The n m r spectra of the inosose phenylosazones (recorded with a Varian A-60 spectrometer, for solutions in methyl sulfoxide, TMS internal standard, δ scale) showed a low-field signal at 12 5–13 p p m (chelate ring-structure), similar to that for D-*myo*-inosose-1 phenylosazone[24] *p*-Dioxane was distilled from a small proportion of lithium aluminum hydride, and was stored in the frozen state until used

*Mention in this article of certain commercial instruments or chemical compounds does not constitute endorsement by the National Bureau of Standards

TABLE I

INFRARED ABSORPTION BANDS (cm^{-1}) OF INOSOSE PHENYLHYDRAZONES AND PHENYLOSAZONES[a]

Assignment	*Compound number*[b]							
	1	2	3	4	5	6	7	8
OH, NH								3575 m
		3480 m						3485 s
		3420 s	3440 sh	3450 s				
	3380 s		3380 s		3390 w	3370 w	3390 s	
		3340 s	3320 s	3300 s	3300 m			3300 s
	3250 s					3280 s	3290 s	3240 s
							3115 m	
C=N	1640 m	1638 m	1657 m	1652 w	1654 w	1680 sh	1688 sh	1690 sh
Phenyl ring	1600 s	1600 s	1605 s	1600 s	1600 s	1600 s	1600 s	1600 s
	1585 w		1588 w					1530 s
		1500 sh		1510 m	1509 s	1510 s	1509 s	1510 m
		1494 m		1490 w	1490 w	1488 m	1490 m	1490 s
N–H (bending)								1575 s
				1562 sh	1560 w	1560 s		1560 s
	1550 s			1555 s	1555 w	1555 sh	1550 s	
		1518 m			1500 s			
Ph–N	1170 s	1170 s	1170 s	1170 s	1170 s	1170 s	1170 s	1170 m
			1149 m	1155 w	1158 sh	1155 w		1150 w
	1130 m	1135 m	1130 s			1130 s	1130 w	1140 m
	1120 m	1120 m		1122 s	1125 s		1121 s	
Fingerprint		1390 w						
region	1340 m		1332 m	1350 w	1340 sh	1340 sh	1340 sh	1330 m
		1312 m	1320 m	1320 m			1325 w	
	1309 m		1310 m	1310 sh		1308 w		1310 sh
		1300 sh		1300 sh	1300 w		1300 m	1300 sh
	1290 w	1270 w	1282 sh					1289 m
	1260 s	1250 s	1258 s	1255 s	1250 s	1250 s	1255 s	1260 s
			1248 sh				1248 sh	
			1230 m			1230 sh	1238 sh	1235 m
	1220 w	1220 w		1215 sh			1228 sh	1218 sh
	1210 w		1200 m		1210 sh	1205 m	1210 w	
	1180 w	1180 w		1190 w			1190 w	1190 w
	1170 s	1170 w	1170 s	1170 s	1170 m	1170 s	1170 s	1170 m
	1150 w	1150 sh	1149 m	1155 w	1158 sh	1155 w	1153 sh	1150 w
	1130 m	1135 m	1130 s			1130 s		1140 m
	1120 m	1120 m		1122 s	1125 m		1121 s	
	1115 m			1109 w		1110 sh		1110 w
	1095 m	1095 s	1100 s		1090 w	1100 s	1105 s	1090 w
		1080 w	1080 m	1079 w		1075 w	1080 sh	1076 s
	1070 s	1070 s			1070 w		1070 s	
		1050 m	1058 s	1050 s	1050 m	1050 w	1050 w	1050 w
						1035 s	1038 s	1040 m
								1030 m
		1020 sh						1025 w
	1010 m		1010 s					1015 m

TABLE I (CONTINUED)

	1000 m						1000 w
990 s	988 w		990 s	995 m	995 w	990 w	990 w
				960 w	970 w	955 s	970 sh
930 m	924 w	935 s		935 w	935 sh	925 m	950 m
	920 m		920 sh	915 m	912 s		
			900 s				900 m
	890 m			890 m	898 w		890 w
880 m	880 sh	880 m			880 w	885 m	885 w
				870 sh	875 w	865 w	870 m
	860 sh		860 w				860 sh
		852 s	850 s				850 w
				830 m	830 m		840 sh
			820 w			825 s	820 sh
		785 s		790 w		780 m	790 m
				772 w	775 m		
750 s	752 s	750 s	750 s	750 s	750 s	750 s	750 s
690 s	695 s	690 s	690 s	690 s	698 s	690 s	695 sh
					680 w		690 s
675 m	672 w	670 w	680 w	665 sh	665 sh	670 w	678 w
							660 sh

[a]Spectra were measured for Nujol mulls [b]Key to compounds 1, *myo*-inosose-2 phenylhydrazone, 2, L-*myo*-inosose-1 phenylhydrazone, 3, DL-*epi*-inosose-2 phenylhydrazone, 4, DL-*myo*-inosose-1 phenylosazone, 5, DL-*epi*-inosose-2 phenylosazone, 6, quebrachitol inosose phenylosazone, 7, pinitol inosose phenylosazone 8 (+)-*proto*-quercitol phenylosazone

Purification of inosose phenylosazones was performed by column chromatography on Florisil* (100–200 mesh, Floridan Co., Philadelphia, Pennsylvania) Generally, a solution of the crude phenylosazone in ethyl acetate was filtered, placed on a column of suitable size, and eluted with 1 l (v/v) 95% ethanol–ethyl acetate or with 95% ethanol More difficultly soluble phenylosazones were dissolved in *p*-dioxane, and eluted with ethanol or 19 l (v/v) ethanol–*p*-dioxane T l c was performed on silica gel G (5 × 20 cm plates, 250 μm thick) for 60 min Separations on columns were monitored by t l c on microscope slides

Preparation of DL-myo-*inosose-1 phenylosazone* (1 and 2) *from* myo-*inositol* — *Procedure A* A precooled solution (3°) of *myo*-inositol (25 g, 139 mmole) and sodium acetate trihydrate (52 g) in 200 ml of water was placed in an ice-bath, and treated with bromine (30 g, 187 mmole). The vessel was stoppered loosely, and the reaction mixture was stirred for 24 h at room temperature (It is important that the temperature of the reaction mixture be kept below 5° for 6–8 h from the beginning of the oxidation) Glacial acetic acid (60 ml) was added, and the excess of bromine was removed by bubbling through the solution a mixture of ethylene and air for 10 to 15 min T l c. of the clear solution [on microcrystalline cellulose[25], 1 mm thick, 5 × 20 cm glass plate, for 60 min with 3·1 l (v/v) butanol–acetic acid–water and the inosose spray[26] (*A*) or the alkaline silver nitrate spray[27] (*B*)] then revealed the presence of two reducing components The faster-moving compound (lilac spot, spray *A*, or dark spot, spray *B*) was found to be *myo*-inosose-2 (major component), the slower-

moving, minor component (yellow spot, spray *B*) is believed to be derived from a diketoinositol To the solution was then added sufficient sodium acetate trihydrate (15 g) to give a negative test with Congo Red paper, the solution was diluted to about 2 5 l with water, and cooled to 10° to 15°, and phenylhydrazine (35 g, 320 mmole) was added. The mixture was stirred for 1 h, and kept for 48 h at room temperature with occasional stirring The orange-red crystals of **1** and **2** that separated were filtered off, washed successively with water, cold 1 1 (v/v) ethanol–water, and cold 95% ethanol, and dried at 50°, weight of crude product 8 3 g Dilution of the filtrate with water (1 liter), and further storage for 36 h, produced an additional crop (4 2 g), total yield 12 5 g (25%), range in several experiments, 11–15 g (22 to 30%)

The crude phenylosazone (**1** and **2**) contained a red impurity*†, R_F 0 96 [2·1 1 (v/v) benzene–acetic acid–butanone], 0 87 (solvent *A*), the osazone had R_F 0 30 The colored material was removed by suspending the crude phenylosazone (10 g) in 100 ml of a freshly prepared, 12% aqueous solution of sodium hydrosulfite ($Na_2S_2O_4$) containing 40 ml of ethanol, and stirring the suspension for 30 min at 80–85° By this time, the red color has disappeared; the suspension was then diluted with water, and cooled The light-yellow phenylosazone was filtered off, washed successively with water and aqueous ethanol, and dried, yield 8 5 g (85%), the product was about 96% pure (i r. spectrum)

The product can be further purified by dissolving it in methanol containing potassium hydroxide (5%) and reprecipitating it by dilution with water, or by recrystallization from aqueous *N*,*N*-dimethylformamide, aqueous 2-methoxyethanol–pyridine, or aqueous ethanol–*p*-dioxane A sample purified by column chromatography (*p*-dioxane–95% ethanol) showed a homogeneous, yellow spot on a thin-layer chromatogram [3 2 1 (v/v) butanone–benzene–acetic acid], R_F 0 41 ±0 01 (solvent *B*), m p 199–201°, (lit [5] m p 199–200°), λ_{max}^{MeOH} 229 (sh) (ε ~15,800), 257 (ε ~23,900), 318 (ε ~11,000), and 398 nm (ε ~20,800)

Procedure B A precooled solution (3°) of *myo*-inositol (100 g, 556 mmole) and sodium acetate trihydrate (208 g) in 800 ml of water was oxidized with bromine (120 g, 750 mmole) for 24 h according to Procedure A The reaction mixture was then treated with acetic acid (100 ml), and excess bromine was removed by addition of aqueous sodium hydrogen sulfite (20% solution, 38 ml). Methanol (500 ml) was then added, and the mixture was diluted to *ca* 4 l with ice-cold water, and treated with phenylhydrazine (100 g), and the mixture was kept for 92 h at room temperature, with occasional stirring The yield of crude, somewhat dark-red **1** and **2** was 31 g (20%), range 29–32 g (18–21%)

In another, otherwise identical, preparation, the excess of bromine was removed by bubbling ethylene–air through for about 55 min; the yield of crude **1** and **2** isolated after 96 h was 41 g (28%)

*The nature of the red impurity present in this and other compounds will be discussed in a subsequent article †Treatment of an ice-cold solution (0 to 5°) with phenylhydrazine sometimes produces a colorless, crystalline compound of unknown structure Recrystallized from ethanol, the compound melted at 226–227°, λ_{max}^{MeOH} 227 and 274 nm Found C, 60 5, H, 5 80

Oxidation of myo-*inositol with hot bromine water* — To a solution of *myo*-inositol (50 g, 278 mmole) in water (200 ml) was added bromine (30 g, 187 mmole) The solution was refluxed (hood) for 90 min, and cooled, and excess bromine was removed by means of sodium hydrogen sulfite. The solution was successively diluted with water (1.8 l) and glacial acetic acid (50 ml), rendered neutral to Congo Red paper with sodium acetate, and treated with phenylhydrazine (35 ml) DL-*myo*-Inosose-1 phenylosazone (**1** and **2**) was obtained in two crops the first crop (3 g) after 36 h, and the second crop (5 g) after an additional 48 h; total yield, 8 g (8.1%).

Preparation of D-myo-*inosose-1 phenylosazone* (**2**) *from* L-*inositol* — A precooled solution (5°) of L-inositol (12 g, 70 mmole) and sodium acetate trihydrate (26 g) in 100 ml of water was treated with bromine (15 g, 93 mmole) by the foregoing procedure, and the mixture was kept for 24 h at room temperature. Glacial acetic acid (30 ml) was added, and the excess of bromine was removed by means of ethylene–air at room temperature Sodium acetate (8 g) was added, and dilution of the solution with methanol (300 ml) and water (700 ml), followed by treatment with phenylhydrazine (20 ml) and occasional stirring, gave a product that was collected in two crops, first crop (3 8 g) after 36 h, and second crop (3 2 g) after dilution with water (1 liter) and further storage (48 h), the total yield of **2** was 7 g (28%) The purified product showed essentially the same R_F value as DL-*myo*-inosose-1 phenylosazone (**1** and **2**) (solvent *B*), m p 206–208° (lit.[3] m p 217°, 210°, lit[24] m p 207–209°), $[\alpha]_D^{23}$ +268 ±10° (24 h), +243 ±5° [*c* 0 01, 1 1 (v/v) ethanol–pyridine], $[\alpha]_D^{23}$ +329 ±20° [*c* 0 01, 1 1 (v/v) ethanol–*p*-dioxane, no mutarotation) [lit[3] $[\alpha]_D^{25}$ +240→214° (1 1 (v/v) ethanol–pyridine)] The product was identical with an authentic sample of D-*myo*-inosose-1 phenylosazone[3]

Preparation of D-myo-*inosose*-1 *phenylhydrazone* (**3**) *from* L-*inositol* — A precooled solution (5°) of L-inositol (12 g, 70 mmole) and sodium acetate trihydrate (26 g) in 100 ml of water was treated with bromine (15 g, 93 mmole) by the foregoing procedure, and the mixture was kept for 24 h at room temperature. The solution was cooled to 5°, and the excess of bromine was removed by means of ethylene–air Sodium acetate (8 g) was added, and the solution was diluted with ice–water (1 liter at 3°), phenylhydrazine (20 ml) was added, and the mixture was stirred vigorously (ice-bath) for 30–45 min The product was separated by filtration, and successively washed with water, ice-cold 50% aqueous ethanol, and ethanol, to give colorless to pale-pink crystals of **3**, yield 5 g (12%) A sample recrystallized from 2-methoxyethanol and then from methanol gave colorless needles, m p 195–197° (lit[14] m p 196–197°); λ_{max}^{MeOH} 278 (ε ~23,200), 302 nm (sh) (ε ~10,350) The product was identical with an authentic sample of D-*myo*-inosose-1 phenylhydrazone[14] The filtrate and washings were combined, diluted with methanol (300 ml) and water to about 700 ml, and kept for 60 h at room temperature, the orange product (6 g, 25 2%) was identical with **2**

Preparation of L-myo-*inosose-1 phenylosazone* (**1**) *from* D-*inositol.* — A precooled (5°) solution of D-inositol (12 g, 70 mmole) and sodium acetate trihydrate (26 g) in 100 ml of water was treated with bromine (15 g, 93 mmole) by the procedure

for oxidation of L-inositol. The yield of **1** was 7.3 g (29%); the product was identical with an authentic sample[3].

Preparation of L-myo-*inosose-1 phenylhydrazone* (**4**) *from* D-*inositol.* — Oxidation of D-inositol (12 g, 80 mmole) with bromine (15 g, 93 mmole) by the procedure described for L-inositol gave **4** (5 g, 12%) and **1** (5 8 g, 24 4%), each product was identical with an authentic sample[3]

*Preparation of 3-*O*-methyl-*myo-*inosose-1 phenylosazone* (**5**) *from quebrachitol* — A precooled solution (5°) of quebrachitol (1-*O*-methyl-*levo*-inositol) (25 g, 120 mmole) and sodium acetate trihydrate (52 g) in 200 ml of water was treated with bromine (30 g, 187 mmole) by the foregoing procedure Acetic acid (80 ml) and sodium acetate (20 g) were added, the solution was cooled to 10°, and the excess of bromine was removed by adding, with stirring, diethylamine (75 ml) Dilution with methanol (300 ml) and water (2 2 1), followed by treatment with phenylhydrazine (35 ml), gave a product that was collected in two crops. first crop (8 8 g) after 36 h, and the second crop (6 2 g) after further dilution with water (1 liter) and storage (48 h), the total yield of crude **5** was 15 g (29%) The crude product contained red impurities, R_F 0 93, 0 87 (solvent *A*), the osazone had R_F 0.66 A product of about 98% purity (i r spectrum) could be obtained as follows the crude phenylosazone **5** was treated with sodium hydrosulfite as described for DL-*myo*-inosose-1 phenylosazone (**1** and **2**) The resulting product (3 g, dry wt) was stirred with warm ethanol (15 ml), and cooled, and the suspension was filtered, to give 2 2 g (73%) of light-yellow needles, m p 191–193° A sample purified by column chromatography (95% ethanol) and recrystallized from aqueous ethanol gave golden-yellow needles, m p 195–197°, $[\alpha]_D^{23}$ −485 ±15° [*c* 0 05, 1 1 (v/v) ethanol–*p*-dioxane], no appreciable mutarotation was observed after 60 min A sample for analysis was dried for 2 h at 140°/0 01 torr.*

Anal Calc for $C_{19}H_{22}N_4O_4$. C, 61 60, H, 5.98, N, 15 12 Found C, 61.80, H, 6.10; N, 15 20

The product showed a single yellow spot on a thin-layer chromatogram, R_F 0 60 ±0 02 (solvent *B*), λ_{max}^{MeOH} 228 (sh) (ε ~16,000), 258 (ε ~24,300), 318 (ε ~11,500), 397 nm (ε ~20,500)

Consumption of periodic acid[3] — Quebrachitol phenylosazone (**5**, 37 mg, 10 mmole) in 60% aqueous ethanol (97 ml) was treated with aqueous periodic acid (3 ml, 536 6mM) at room temperature An aliquot (10 ml) was withdrawn and analyzed every 15 min (6 determinations) The excess of periodic acid was determined with sodium arsenite; 2.80 ml of the titrant (100.3mM) was consumed, the average consumption of periodic acid per mole of osazone **1** was 2 06 moles After 1 week, overoxidation had occurred, indicating that the methoxyl group had undergone scission

*Preparation of 5-*O*-methyl-*myo-*inosose-1 phenylosazone* (**6**) *from pinitol* — A precooled solution (5°) of pinitol (5-*O*-methyl-*dextro*-inositol) (12 5 g, 60 mmole) and sodium acetate trihydrate (2 6 g) in 100 ml of water was treated with bromine

*Dried at 25°/0 1 torr, a hemihydrate was obtained

(15 g, 935 mmole) by the foregoing procedure. Glacial acetic acid (35 ml) and sodium acetate (8 g) were added, the solution was cooled to 10°, and the excess of bromine was removed by bubbling ethylene–air through, with stirring, for 10 min. The clear solution was diluted with water (700 ml) and methanol (300 ml), and treated with phenylhydrazine (20 ml) The solution was kept for 96 h at room temperature, and the product was collected in two crops, first crop (7–8 g) after 48 h, and the second crop (1.6 g) after dilution with water (1 l), the total yield of crude **6** was 9.4 g (31%). The crude product contained a considerable proportion of decomposition products; R_F 0 96, 0 93 (red), 0 84 (orange-yellow) (solvent *A*) The osazone had R_F 0.68. The crude product (7 g) was treated with sodium hydrosulfite (10% solution, 70 ml) at 90° to give the phenylosazone **6**, 4.5–5.5 g (64–78%). This product was further purified by column chromatography. a solution of **6** (1 g) in ethanol (100 ml) was passed through a column of Florisil (4 × 35 ml) and eluted with 95% ethanol The first zone (orange-yellow) was discarded; the second zone (light yellow) was collected and concentrated to dryness, and the resulting solid was recrystallized from aqueous ethanol to give **6** as light-yellow crystals; yield 0 2 g (20%), m p. 206–207° (with darkening at 204°), $[\alpha]_D^{23}$ −507 ±25° (3 min) (rapid mutarotation) → 0 00° (63 min) → +88 ±5° (123 min) → +130 ±15° [final, 24 h, *c* 0 01, 1·1 (v/v) ethanol–*p*-dioxane] A sample* for analysis was dried for 2 h at 140°/0 1 torr

Anal Calc. for $C_{19}H_{22}N_4O_4$ C, 61 60, H, 5.98; N, 15 12 Found C, 61 84; H, 6 12, N, 15.15.

The product showed a single, yellow spot on a thin-layer chromatogram, R_F 0 58 ±0 02 (solvent *B*), λ_{max}^{MeOH} 230 (sh) (ε ~15,300), 258 (ε ~24,700), 321 (ε~11,300), 396 nm (ε ~20,000) One mole of **6** consumed 0 97 mole of periodic acid

Preparation of DL-epi-*inosose-2 phenylosazone* (**8**) *from* DL-epi-*inosose-2 phenylhydrazone* — A solution of DL-*epi*-inosose-2 phenylhydrazone[16] (colorless needles from 2-methoxyethanol, m p 188–190°) (0 5 g, 6 mmole) in methanol (200 ml) was diluted with water (50 ml) and treated with phenylhydrazine (2 ml) and acetic acid (10 ml) The reaction mixture (in a loosely stoppered bottle) was kept for 24 h at room temperature, water (30 ml) was then added and the solution was kept for 24 h The mixture was then transferred to a beaker, and allowed to evaporate slowly (hood) for 36–48 h An orange-red solid (0 2 g) was collected, treated with aqueous sodium hydrosulfite (10 ml), and purified by column chromatography (*p*-dioxane–95% ethanol), orange-yellow fractions [λ_{max}^{MeOH} 230 (sh), 305, 328, and 396 nm] were discarded; a slow-moving, yellow zone was collected, and evaporated to dryness, and the resulting solid was recrystallized from aqueous ethanol–*p*-dioxane to give **8** as yellow crystals; yield 0 05 g (7 6%), m p. 202–204°, R_F 0 38 ±0 01 (solvent *B*); λ_{max}^{MeOH} 229 (sh) (ε ~15,800), 258 (ε ~23,900), 318 (ε ~11,900), 398 nm (ε ~21,200). A sample for analysis was dried for 2 h at 140°/0 1 torr

*A sample dried at 25°/0 1 torr contained 0 5 molecule of water of crystallization per molecule Calc for $C_{19}H_{22}N_4O_4$ 0 5 H_2O C, 60 14, H, 6 10, N, 14 80 Found C, 60 20, H, 5 95, N, 14 90

Anal Calc. for $C_{18}H_{20}N_4O_4$: C, 60.65, H, 5.65, N, 15 72 Found C, 60 80, H, 5.75, N, 15.60.

One mole of **8** consumed 3 12 moles of periodic acid

Preparation of (+)-proto-*quercitol** *phenylosazone* (**7**) *from acorns* — Acorn flour (1 kg), from dried acorns crushed in a ball mill, was soaked in distilled water (2 l) for 48 h at room temperature, with occasional stirring The suspension was filtered, and the solid was washed with water (600 ml) The dark-brown filtrate and washings were combined, lead(II) acetate (35 g) in 20% acetic acid (120 ml), and Celite (50 g) were added, and the suspension was stirred for 30 min and filtered The filtrate and washings were combined (about 3 l), and treated with hydrogen sulfide, and the suspension was filtered through a layer of Celite The solid was washed with water, and the filtrate and washings were combined (about 3 2 l), treated with sodium acetate trihydrate (200 g) and glacial acetic acid (150 ml), and cooled to 10° Bromine (90 g) was added, and the mixture was stirred for 48 h at room temperature To the mixture was added sodium acetate (100 g), the solution was cooled to 10°, and the excess of bromine was removed with diethylamine (50 ml). Phenylhydrazine (100 ml) was added, and the suspension was stirred for 2 h, and kept for 96 h at room temperature The product was collected in three crops. the first crop (29 g) after 48 h, the second crop (25 g), following dilution with water (1 liter), after an additional 24 h; and the third crop (3 g) after an additional 24 h The total yield of light-yellow **7** was 57 g (20%)

A sample recrystallized from aqueous ethanol–*p*-dioxane had m p 201–203° (dec.) (lit [28] m p 199–200°), $[\alpha]_D^{23}$ +102 ±5° [*c* 0 01, 1.1 (v/v) ethanol–*p*-dioxane] [lit [28] $[\alpha]_D^{25}$ +65° (1 1 (v/v) ethanol–pyridine)], R_F 0 28 ±0 01 (solvent *B*), λ_{max}^{MeOH} 229 (sh) (ε ~15,100); 255 (ε ~21,300), 308 (ε ~11,800), and 393 nm (ε ~18,600) The product was identical with an authentic sample[6 28] of **7**

Potassium hypobromite oxidation of myo-*inositol, isolation of* DL-xylo-*pentahydroxy-2-cyclohexen-1-one* — A precooled solution (5°) of *myo*-inositol (25 g, 139 mmole) and potassium acetate (37 g) in 200 ml of water was treated with bromine (30 g, 187 mmole), and stirred for 36 h at room temperature The excess of bromine was removed by means of air–ethylene, and the solution was treated with solid potassium carbonate, and stirred for about 20 min at 90° until it remained permanently basic (pH 9–10) The dark solution was then concentrated until crystallization started, and the suspension was cooled and filtered. The filtrate was concentrated to about 20 ml, cooled, nucleated, and kept for ten days in a refrigerator, with occasional shaking and scratching After separation of a small quantity of blue crystals of potassium rhodizonate, the filtrate deposited light-yellow crystals, yield 1 2 g (4%) After recrystallization from aqueous acetone, the product exhibited the properties of an authentic sample of the potassium salt of DL-*xylo*-pentahydroxy-2-cyclohexen-1-one[19]

*(+)-*proto*-Quercitol is named (+)-1-deoxy-*muco*-inositol by the Fletcher–Anderson–Lardy system

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