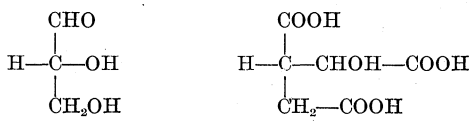
chemists have agreed on a correlation rule with respect to certain of the groups surrounding the asymmetric centers in formulae A and B.



FORMULA A
D-Glyceraldehyde

FORMULA B
Isocitric Acid

There would be little doubt that —COOH in B is to be correlated with —CHO in A, and —H is common to both. However, it is doubtful that all would agree that the group —CHOH—COOH in B should be correlated with the group —OH in A and, further, that the group —CH_2—COOH in B should be correlated with the group —CH_2OH in A. In the absence of a widely accepted rule that permits such correlations to be made, to say nothing of the absence of any direct chemical evidence upon the actual configuration of the β-carbon atom of this substance, it is impossible to use a capital letter nomenclature for isocitric acid which signifies the configuration of the asymmetric center at the β-carbon atom; that is, neither of the names D_g-isocitric acid nor L_g-isocitric acid is currently admissible.

In view of the fact that the isomer of isocitric acid found in plant leaves is dextrorotatory in water solution (*4, 5*), the substance may be designated *d*-isocitric acid where the italic *d* has its original significance *dextrorotatory*.[1] Nevertheless, *d* and *l* have been ambiguous ever since they were first used by Fischer to denote configuration rather than direction of rotation in the carbohydrate field, and this practice was extended to amino acids by Wohl and Freudenberg. Accordingly, where one wishes to avoid all possibility of being misunderstood, the name should be written *dextro*-isocitric acid or (+)-isocitric acid, the latter method of expression being admissible under present amino acid rules and in common use, especially in papers of British origin.

The lactone that is readily formed when a water solution of *dextro*-isocitric acid is evaporated to a sirup is strongly levorotatory (*4*) and should be named *levo*-isocitric lactone, or (−)-isocitric lactone or even *l*-isocitric lactone, provided it is made clear that the prefix is being used in its original sense. However, the greatest possibility of confusion in naming isocitric acid arises from the fact that the ammonium molybdate complex compound of *dextro*-isocitric acid has an extraordinarily high *levorotation* (*6–9*), and it is customary to employ the molybdate complex in examining the rotation of solutions of isocitric acid. There is thus a tendency to designate the naturally occurring substance *l*-isocitric acid (or (−)-isocitric acid) or its salt as *l*-isocitrate. A number of papers have appeared in which this error occurs, and care must therefore be taken in reading the literature of this substance.

[1] Although the use of the prefixes *d-* and *l-* is not recognized, under the rules approved by the American Chemical Society for carbohydrate nomenclature or for amino-acid nomenclature, to indicate the direction of rotation of a solution of the substance, they are widely and entirely properly employed for this purpose in other fields; e.g., for optically active amines, alcohols, hydrocarbons, vitamins, alkaloids, etc. The α-hydroxy acids are chemically and metabolically closely allied with both amino acids and carbohydrates, and their nomenclature at the present time is in a state of flux. Divergent opinions have, for example, been expressed as to whether *dextro*-tartaric acid should be named as a D compound or as an L compound. Unusual care should therefore be taken in naming this group of substances.

References

1. CRANE, E. J. *Chem. Eng. News*, **25**, 1364 (1947).
2. *Ibid.*, **26**, 1622 (1948).
3. BREWSTER, P., *et al. Nature*, **166**, 178 (1950).
4. BRUCE, W. F. *J. Am. Chem. Soc.*, **57**, 1725 (1935).
5. PUCHER, G. W., and VICKERY, H. B. *J. Biol. Chem.*, **145**, 525 (1942).
6. MARTIUS, C. *Z. physiol. Chem.*, **257**, 29 (1938).
7. PUCHER, G. W. *J. Biol. Chem.*, **145**, 541 (1942).
8. KREBS, H. A., and EGGLESTON, L. V. *Biochem. J.*, **37**, 334 (1943).
9. EGGLESTON, L. V., and KREBS, H. A. *Ibid.*, **45**, 578 (1949).

The Reported Adrenergic Blocking Action of β-Haloethylammonium Compounds

James F. Kerwin, Grisella C. Hall, Mark Nickerson,[1] William S. Gump,[2] Richard A. McLean, Edwin J. Fellows, and Glenn E. Ullyot

Research Division, Smith, Kline & French Laboratories, Philadelphia, Pennsylvania

Recently Nyman and co-workers (*1–3*) have reported the preparation of two quaternary derivatives of N,N-dibenzyl-β-chloroethylamine (Dibenamine[3]). One of these, dibenzyl-β-chloroethyl ethylammonium bromide, was claimed (*3*) to be an effective adrenergic blocking agent and, furthermore, to exert its full effect within 5 min after intravenous injection. In a few other instances quaternization of β-haloethylamines has been reported to produce active salts (*4*). The materials tested were noncrystalline, however, and therefore of questionable purity, and, in each case, the potency was less than that of the corresponding tertiary amine.

In our experience, the ability of β-haloethylamines of the Dibenamine type to undergo intramolecular alkylation with the formation of ethylenimonium ions appears to be of prime importance for adrenergic blocking action. Also, the rate of onset of the blocking effect seems to be dependent upon the reactivity of the halogen. Further alkylation of the amino group would be expected to produce inactive compounds, since the nitrogen is unable to participate in the initial cyclization reaction unless dissociation to a tertiary amine were to occur under physiological conditions. As further evidence for our hypothesis that imonium ions are the active intermediate, we prepared a num-

[1] Department of Pharmacology, University of Utah College of Medicine, Salt Lake City.
[2] Givaudan-Delawanna, Inc., Delawanna, N. J.

[3] Smith, Kline & French trade-mark.

TABLE 1

Dibenzyl-β-haloethyl alkylammonium halides

$(C_6H_5CH_2)_2\overset{+}{N}(R)CH_2CH_2X\ Y^-$

R	X	Y	mp (degrees)	Analyses (%) Calcd C	Calcd H	Calcd Y	Found C	Found H	Found Y
CH_3	Cl	I	154.5–155.5	50.82	5.27	31.59	50.87	5.49	31.66
CH_3	Br	Br	164	51.15	5.30	20.02	51.18	5.04	20.23
C_2H_5	Cl	Cl	177–178	66.66	7.15	10.94	66.71	7.35	11.06
C_2H_5	Br	Br	154.5–156.5	52.32	5.61	19.34	52.31	5.78	19.36

ber of quaternary derivatives of β-haloethylamines, all of which were found to be devoid of the blocking action typical of Dibenamine and other β-haloethylamines. This apparent contradiction between our results and those of the Swedish workers prompted us to repeat their preparation.

When dibenzyl-β-chloroethylamine was heated with an excess of ethyl bromide in butyl alcohol, as directed by Nyman and Plantin (*2*), we obtained a solid which melted at 178° to 179.5° (d) after one recrystallization from alcohol and ether (reported [*2*] mp, 178°–180° d). The elementary analysis (C, 50.04; H, 5.23; Br^-, 21.11; total Br, 39.23; Cl, 0.87) did not correspond to the percentages calculated for the expected dibenzyl-β-chloroethyl ethylammonium bromide which requires C, 58.63; H, 6.29; Br^-, 21.67; Cl, 9.62. Treatment of an aqueous solution of the product with sodium bicarbonate precipitated an oily tertiary amine free base. This behavior and the elementary analysis indicated that the product was largely dibenzyl-β-bromoethylamine hydrobromide (Calcd for $C_{16}H_{19}NBr_2$: C, 49.89; H, 4.76; Br^-, 20.75; total Br, 41.50) containing about 8% of dibenzyl-β-chloroethylamine hydrobromide. A second recrystallization of the product from alcohol, with a recovery of 86%, did not affect the melting point (177.5°–179° d), but the sample no longer contained chloride, and the bromine analysis corresponded to that calculated for pure dibenzyl-β-bromoethylamine hydrobromide (Found: total Br, 41.35). A mixture of this material or that obtained after the first recrystallization with authentic dibenzyl-β-bromoethylamine hydrobromide (*5*) (mp, 177°–179° d) showed no depression in melting point. Thus, the activity and rapid onset of action of Nyman and Plantin's product are not unexpected and, indeed, agree closely with the properties observed by us for authentic dibenzyl-β-bromoethylamine hydrobromide.

Similarly, the reaction of dibenzylaminoethanol and ethyl bromide, also described by Nyman and Plantin (*2*), produced dibenzylaminoethanol hydrobromide and not a quaternary derivative. This was demonstrated by analysis, comparison of melting points, and conversion of the product into the known free base (*6*) with sodium bicarbonate.

In other attempts to prepare quaternary derivatives of dibenzyl-β-chloroethylamine we have noticed that direct interaction of the base with alkylating agents forms either salts of the tertiary amine, often with halogen interchange, or difficultly separable mixtures containing salt of the tertiary amine, quaternary derivatives, and, occasionally, dimerization products. Reexamination of the crude resinous "quaternary salts" tested by one of us (*4*) revealed that the materials were such mixtures with sufficient tertiary amine present to account for the reported activity.

A less direct but preferred method for preparing pure quaternary derivatives of Dibenamine consists of the following steps:

$$C_6H_5CH_2X + C_6H_5CH_2NHCH_2CH_2OH \longrightarrow (C_6H_5CH_2)_2\overset{+}{N}(R)CH_2CH_2OH\ X^-$$

I

$$\xrightarrow{SOX_2} (C_6H_5CH_2)_2\overset{+}{N}(R)CH_2CH_2X\ X^- \qquad R = CH_3,\ C_2H_5;\ X = Cl,\ Br$$

II

The intermediate alcohols (I) were crystalline solids readily soluble in water or basic solution. Treatment with thionyl chloride or thionyl bromide in chloroform solution produced the desired β-haloethylammonium salts. In most cases the products were easily crystallizable solids which were readily purified by recrystallization from alcohol and ether. Dibenzyl-β-chloroethyl methylammonium chloride, however, persisted as an oil and was converted into the crystalline iodide by addition of potassium iodide to an aqueous solution of the crude chloride. The properties of these salts are listed in Table 1.

When the compounds in Table 1 were tested for adrenergic blocking action in cats after intravenous administration according to methods previously described (*7*) they were found to be without Dibenamine-like blocking ability in toxic doses of 10–20 mg/kg.

We were unable to obtain dibenzyl-β-chloroethyl methylammonium methosulfate in solid form, but the crude salt could be purified in the following manner. The resinous material obtained by heating dibenzyl-β-chloroethylamine and dimethyl sulfate in refluxing benzene was treated with acetone and filtered to remove a dimerization product. The acetone soluble material was dissolved in water, made slightly alkaline and extracted with ether to remove tertiary amine. The concentration of quaternary salt in the aqueous layer could be approximated by adding potassium iodide to an aliquot and weighing the crystalline, water-insoluble dibenzyl-β-chloroethyl methylammonium iodide. When

this solution of methosulfate was injected, no reversal of the pressor effect of epinephrine was detected. It can be presumed then that the unpurified dibenzyl-β-chloroethyl methylammonium methosulfate previously reported to be active (*4*) in reality contained an appreciable amount of tertiary amine.

The effect of quaternization on the reactivity of the β-chloroethyl compounds listed in Table 1 was determined by measuring the increase in halide ion when the compounds were dissolved in 70% aqueous alcohol containing sodium bicarbonate. No appreciable increase in halide ion concentration was noted over a 24-hr period, whereas, under the same conditions, the organic chloride of Dibenamine is 50% converted into chloride ion in 40 min. We feel that this difference between the tertiary amines and the quaternary salts adequately explains the inactivity of the latter and provides further support for the theory that the adrenergic blocking action of Dibenamine is dependent upon ethylenimonium ion formation in the body. Additional details of this work will be reported elsewhere.

References

1. NYMAN, E. *Nature,* **164,** 269 (1949).
2. NYMAN, E., and PLANTIN, L. *Acta Physiol. Scand.,* **19,** 1 (1949).
3. NYMAN, E., and OSTLAND, E. *Ibid.,* 4 (1949).
4. NICKERSON, M., and GUMP, W. S. *J. Pharm. Exptl. Therap.,* **97,** 25 (1949).
5. GUMP, W. S., and NIKAWITZ, E. J. *J. Am. Chem. Soc.,* **72,** 1309 (1950).
6. EISLEB. U. S. Patent 1,949,247 (1934).
7. KERWIN, J. F., *et al. J. Am. Chem. Soc.,* **72,** 940 (1950).

Differentiation of Minimus Type *C. diphtheriae* by Slow Fermentation of Dextrose

Elizabeth I. Parsons and Martin Frobisher, Jr.

Communicable Disease Center,*[1] *Public Health Service, Federal Security Agency, Atlanta, Georgia

A distinguishing character of the *minimus* type of *C. diphtheriae* is failure to ferment dextrose promptly or at all, *when first isolated.* After repeated transfer upon artificial media, *minimus* type organisms will manifest normal fermenting ability and will attack dextrose as readily as other strains.

Several workers to whom we have sent cultures of *minimus* type *C. diphtheriae* report that, in their hands, these organisms fermented dextrose as readily as the *mitis* type. As a result of correspondence regarding technical details, we now believe it worth while to give explicit information regarding the method requisite to demonstrate this difference.

The medium used is Difco heart-infusion broth with a pH of 7.8 after autoclaving. Brom-cresol-purple is used as indicator. To each 3.0 ml of this medium, in "Wassermann tubes," is added, aseptically, 0.3 ml of a 10% sterile aqueous solution of chemically pure dextrose. This may be sterilized either by filtration or by autoclaving at 10 lb for 10 min.

[1] From Laboratory Services, Chamblee, Ga.

The organisms to be tested are grown in the same heart-infusion broth as above, without the dextrose or indicator, for 48–72 hr. The dextrose medium is inoculated with one or two drops of this culture, using a capillary pipette or a wire loop. No serum is used in any of the media. Incubation is at 37° C. A *mitis* strain will ferment the dextrose in 24–48 hr, whereas a true *minimus* strain, upon primary isolation, will not ferment before 8–10 days, if at all.

The Histochemical Demonstration of Succinic Dehydrogenase[1, 2, 3]

Arnold M. Seligman and Alexander M. Rutenburg[4]

Yamins Laboratory for Surgical Research, Beth Israel Hospital,* and *Department of Surgery, Harvard Medical School, Boston

Succinic dehydrogenase plays a vital role in respiratory processes of most living cells and forms a link in the chain of reactions concerned with the oxidation of lipids, carbohydrates, and proteins (*1*). In view of the relative importance of this enzyme in physiological processes, it was considered worth while to develop a method for the histochemical demonstration of succinic dehydrogenase in tissue sections.

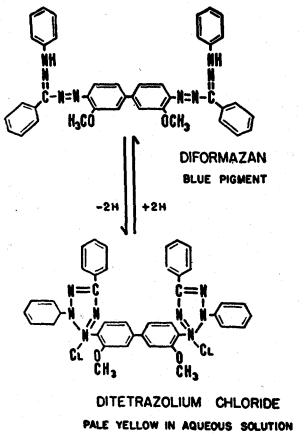


FIG. 1.

The preparation and use of a ditetrazolium chloride[5] (BT, Fig. 1) in the demonstration of specific dehydrogenase activity in extracts of tissue homogenates have been described previously (*2*). In the presence of

[1] This investigation was supported by a research grant from the National Cancer Institute, National Institutes of Health, USPHS, in part by a grant from the Massachusetts Division of the American Cancer Society, and in part by an institutional grant to Harvard University from the American Cancer Society.

[2] Acknowledgments are due Irving Siegel, Myron Milden, and Selma Rutenburg for technical assistance.

[3] Photomicrographs by Leo Goodman.

[4] Research fellow in surgery.

[5] The reagents for this method may be obtained from Dajac Laboratories, Monomer-Polymer, Inc., 3430 W. Henderson St., Chicago 18, Ill.