methane, ethanolamine, triethanolamine, and ammonia gas, at very low concentrations, formed saponification products which resulted in clear, stable emulsions. Three cationic agents, cetylpyridinium chloride, stearyltrimethylammonium chloride, and stearyldimethylbenzylammonium chloride, likewise produced stable emulsions of this system.

Method I, in which the surfactant was added to the glycerin, was effective in all of the cases mentioned; whereas Method II, in which the surfactant was added to the oil, was effective in only a few isolated cases—namely, with ammonia, triethanolamine, and stearyltrimethylammonium chloride. All of the emulsions formed were of the oil-in-glycerin type, with the exception of emulsions containing sodium stearate or stearyltrimethylammonium chloride.

REFERENCES

(1) Monte-Bovi, A. J., J. Am. Pharm. Assoc., **11**, 107 (1950).
(2) Applewhite, R. W., Buckley, A. P., and Nobles, W. L., *ibid.*, **15**, 164(1954).
(3) Leyden, A. F., Pomerantz, E., and Bouchard, E. F., This Journal, **45**, 771(1956).
(4) Goodhart, F. W., and Martin, A. N., *ibid.*, **51**, 50 (1962).
(5) Gregg, R. M., and Zopf, L. C., *ibid.*, **40**, 390(1951).
(6) Sindar Corp., Technical Bulletin H-6, 1952.
(7) Zirkle, M., "Selected Pharmaceutical Research References," Smith Kline and French Laboratories, Philadelphia, Pa., Manuscript No. 21, 1957.
(8) Wagner, J. G., This Journal, **50**, 359(1961).
(9) Eikholt, T. H., and White, W. F., *Drug Std.*, **28**, 154(1960).
(10) Barr, M., This Journal, **51**, 395(1962).
(11) Kolthoff, I. M., and Stricks, W., *J. Phys. Colloid Chem.*, **52**, 915(1948).
(12) McBain, J. W., and Richards, P. H., *Ind. Eng. Chem.*, **38**, 642(1946).
(13) McBain, M. E. L., and Hutchinson, E., "Solubilization and Related Phenomena," Academic Press, New York, N. Y., 1955.
(14) Higuchi, W. I., and Misra, J., This Journal, **51**, 455(1962).
(15) Ross, S., *J. Colloid Sci.*, **6**, 497(1951).
(16) Arkin, L., and Singleterry, C. R., *J. Am. Chem. Soc.*, **70**, 3965(1948).
(17) Mathews, M. B., and Hirschhorn, E., *J. Colloid Sci.*, **8**, 86(1953).
(18) Peri, J. B., *J. Am. Oil Chemists' Soc.*, **35**, 110(1958).
(19) Aebi, C. M., and Wiebush, J. R., *J. Colloid Sci.*, **14**, 161(1959).
(20) Becher, P., *J. Phys. Chem.*, **64**, 1221(1960).
(21) Hall, N. A., personal communication.

Comparative Activities of Maleyl, Fumaryl, and Succinyl Dicholine

A Correction of the Literature

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A reinvestigation of the synthesis and structure of "dicholine maleate" reported by an earlier worker has shown that the *cis*-double bond was isomerized to the *trans*-form. An alternate synthesis for dicholine maleate has been repeated and has been found to give rise to the correct structure. Contrary to literature reports, there are marked differences in biological potency between maleyl and fumaryl dicholines compared with succinyl dicholine.

In considering structure-activity relationships of succinyl dicholine, conformations of the succinic acid portion of the ester assume some importance. If one considers possible conformations of a succinate ester, it would be expected that a staggered conformation of the acid would be favored over eclipsed or gauche forms. Inspection of Dreiding models of maleic and fumaric acids reveals that the carboxyl groups of these acids are oriented in space in a manner which closely resembles the orientation in space of the eclipsed and the staggered conformations, respectively, of succinic acid. The question then arises as to whether the staggered conformation would be favored for adsorption at a biological receptor surface.

Dicholine esters of maleic and fumaric acids offer special interest in that they are unsaturated analogs of succinyl dicholine. The double bond confers some degree of rigidity upon the acid portion of the system; thus the quaternary groups are held in a somewhat fixed, rigid relationship to each other, being relatively close together in the *cis*-maleate ester, and relatively far apart in the *trans*-fumarate ester. If the concept that both quaternary heads of succinyl dicholine attach in some specific manner to receptor sites and/or to "anchoring" sites is valid, there should be considerable difference in biological potency between the dicholine esters of the two isomeric unsaturated acids. Cavallito and Gray (1) stated that the fumaryl and the maleyl esters of choline

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are equally and fairly active. Brücke (2) cited literature reports that fumaryl dicholine is approximately one-half as potent as succinyl dicholine in the rabbit head-drop test. This author, while listing maleyl dicholine in his tables, recorded no biological data for it. Glick (3) reported synthesis of maleyl dicholine by condensing maleic anhydride with ethylene bromohydrin using heat but no solvent or catalyst, followed by treatment of the resulting bis-(2-bromoethyl) maleate with an excess of trimethylamine. Glick did not report test data of succinyl dicholine-like activity for this ester; hydrolysis rates for the compound which were reported by Glick were extremely similar to those reported by other workers for analogous fumarate esters. Synerholm and Hartzell (4) reported the preparation of bis-(2-chloroethyl) maleate by reacting 2-chloroethanol with maleic anhydride. When the experiments of Glick and of Synerholm and Hartzell leading to bis-(2-haloethyl) maleates were repeated in this laboratory, products were obtained which corresponded in melting points and in other physical characteristics to those reported by the original workers; however, these products were identical (evidenced by infrared spectral studies and by mixed melting points) to fumarate esters which were prepared in an unumbiguous manner. Therefore, it would appear that the hydrogen halide, formed in small amounts during the reaction of maleic anhydride with the halohydrin, catalyzed isomerization of the *cis* double bond to the more stable *trans*. It is well established (5) that maleates are rapidly converted to fumarates in the presence of hydrohalic acids. It would appear that Glick's "maleyl dicholine" was actually fumaryl dicholine.

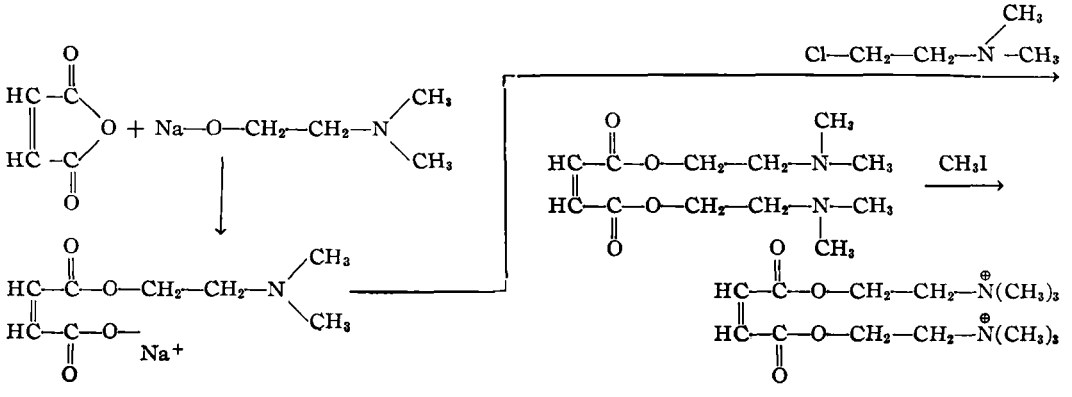
The only other recorded synthesis of maleyl dicholine is that of Christiansen and co-workers (6), who reported no data to support the structure of the material claimed to be maleyl dicholine. Hence, their synthesis was repeated in this laboratory as outlined

Mayo and Walling (7) have stated that primary and secondary amines will cause isomerization of maleates to fumarates. However, these authors pointed out that tertiary amines (except for pyridine) are known not to effect this isomerization. Hence, it was expected that no change in the configuration about the double bond would occur in the preparation of the bis-(2-dimethylaminoethyl) ester of maleic acid. Confirmation of this was obtained when the infrared spectrum of bis-(2-dimethylaminoethyl) fumarate (prepared from bis-(2-bromoethyl) fumarate and dimethylamine) was found to be significantly different from that of bis-(2-dimethylaminoethyl) maleate prepared by the method of Christiansen and co-workers (6).

Application of classical methods to prove that treatment of bis-(2-dimethylaminoethyl) maleate with excess methyl iodide did not result in isomerization of the double bond and that the final product of the reaction sequence was indeed dicholine maleate led to inconclusive results. When dicholine maleate, prepared in this laboratory by the method of Christiansen and co-workers (6), was hydrolyzed with base, only fumaric acid could be isolated following acidification. This result is not necessarily significant, since the iodide ions present in the solution will be converted into hydriodic acid on acidification of the reaction mixture with sulfuric acid. In addition, air oxidation could form small amounts of free iodine which is known (5) rapidly to isomerize maleic to fumaric acid. The fact that the acidic solution from which the fumaric acid was isolated assumed the color of a dilute solution of iodine supports this contention.

A comparison of the decomposition points of an authentic sample of dicholine fumarate and of dicholine maleate prepared by Christiansen's method revealed that the two products melted with decomposition at the same temperature (252–253°) when determined under identical conditions, individually, and when mixed. Generally, these data are taken as proof that two samples from different sources are identical. However, it has been reported (8) that maleic acid is converted to the higher melting fumaric acid when it is heated slightly above its melting point; hence, it could be argued that at such high temperatures, dicholine maleate might be expected to isomerize to dicholine fumarate.

Infrared spectroscopy would not be expected to furnish conclusive results; the dicholine esters are completely insoluble in solvents used for infrared studies. Hence, it would be necessary to determine



their spectra in the solid state. Differences in spectra of closely related compounds taken in the solid state can be due to differences in crystalline forms rather than to differences in molecular structure. Stafford and his co-workers (9) have stated that the absorption maxima of maleates and of fumarates in the ultraviolet occur at the same wavelength (210 mμ region), but that the extinction coefficient of fumarates is greater than that of maleates. When the ultraviolet spectra of dicholine maleate diiodide and of dicholine fumarate diiodide were recorded in methanol, an absorption maximum appeared at 219 mμ, with an extinction coefficient of 15,000 in both cases. This maximum is probably not due to the unsaturated chromophore but to the iodide ion. Knight and co-workers (10) have reported that the ultraviolet spectrum of tetramethylammonium iodide contains an absorption maximum at 226 mμ with an extinction coefficient of 13,300. These workers attributed this absorption to iodide ion.

Support for the structure of dicholine maleate was obtained by paper chromatographic analysis (see Table I). Goldenberg and Spoerri (11) found that esters of dicarboxylic acids in which the two ester groups were in close proximity, such as diesters of maleic and phthalic acids, reacted more slowly with aqueous alkaline hydroxylamine to give hydroxamic acids than did diesters of fumaric acid or diesters in which the ester groups were separated by more than two carbons. Also, a much less intense color was produced in the case of maleic and phthalic acids on addition of ferric chloride reagent. The fact that the di-quaternary compound arising from bis-(2-N,N-dimethylaminoethyl) maleate failed to develop a color when paper chromatograms were treated with the hydroxylamine–ferric chloride reagent was interpreted as evidence that no isomerization of the double bond had occurred.

The differences which were noted in biological activities of the dicholine esters of maleic and fumaric acids further substantiated the structure of the dicholine maleate prepared by the method of Christiansen and co-workers.

Schilling and Pedersen (14) reported that maleyl dicholine prepared by Christiansen and co-workers (6) had some ability to paralyze skeletal muscle. However, these workers did not compare similar dose levels of maleyl dicholine and of succinyl dicholine, and they did not report tests on fumaryl dicholine. A search of the literature has not revealed that any group has prepared authentic maleyl dicholine and carried out parallel tests on it, on fumaryl dicholine, and on succinyl dicholine. The importance of steric factors in structure-activity relationships of quaternary compounds indicates the desirability of concurrent tests of these three esters.

TABLE I.—PAPER CHROMATOGRAPHIC ANALYSIS OF DICHOLINE MALEATE AND OF DICHOLINE FUMARATE[a]

Dicholine Ester	Reaction to Iodine (12)	Reaction to Hydroxylamine-ferric chloride (13)	R_f Value
Maleate	+	−	0.18
Fumarate	+	+	0.18

[a] The paper chromatographic system used was that of Augustinsson, K. B., and Grahn, M., *Acta Chem. Scand.*, 7, 906(1953).

The threshold dose of succinyl dicholine, determined in 13 cats, was approximately 0.04 to 0.05 mg./Kg., with a duration of action between 2 and 14 minutes.

Fumaryl dicholine produced a blockade of skeletal muscle contraction at a threshold dose of approximately 0.12 mg./Kg. Doses ranging between 0.06 and 2.32 mg./Kg. were administered to four cats. The compound did not produce significant changes in blood pressure below this threshold dose; however, doses above 0.2 mg./Kg. produced pressor effects and marked respiratory depression.

Maleyl dicholine was much less effective than either fumaryl dicholine or succinyl dicholine, and the threshold dose was approximately 2.0 mg./Kg. Doses ranging between 0.1 and 4.38 mg./Kg. were administered to four cats. The compound also produced transient hypotensive effects in the lower dose range (0.1 to 2.0 mg./Kg.) and marked hypotensive effects in doses in excess of 2.0 mg./Kg.

EXPERIMENTAL[1]

Chemical

Bis-(2-bromoethyl) Fumarate.—*A. From Fumary Chloride.*—Sodium dried, thiophene-free benzene (125 ml.), 17 Gm. (0.175 mole) of anhydrous reagent grade potassium carbonate, and 20 ml. (34.5 Gm., 0.276 mole) of 2-bromoethanol were mixed in a 500-ml. three-neck flask equipped with a mechanical stirrer, dropping funnel, and a condenser topped with a drying tube. A 14.5-Gm. (0.095 mole) quantity of fumaryl chloride (15) in 125 ml. of benzene was added over a 15-minute period to the rapidly stirred contents of the flask. Stirring was continued for 3 to 4 hours; then 100 ml. of chloroform was added, and the insoluble inorganic material was removed by filtration. The filtrate was washed successively with 100 ml. of water, two 100-ml. portions of saturated sodium bicarbonate solution, and 100 ml. of water. Removal of the solvent under reduced pressure (after drying with sodium sulfate) gave 22.1 Gm. (70%) of a white solid, which was recrystallized twice from Skelly B; m.p. 66–67°.

Anal.—Calcd. for $C_8H_{10}Br_2O_4$: C, 29.12; H, 3.05; Br, 48.44. Found: C, 29.76; H, 3.17; Br, 47.45.

B. From Maleic Anhydride.—A 10.65-Gm. (0.11 mole) quantity of freshly sublimed maleic anhydride (or Eastman white label grade, without sublimation) and 14.3 ml. (25 Gm., 0.2 mole) of 2-bromoethanol were placed in a 250-ml. round bottom flask equipped with a condenser and drying tube. The mixture was heated at 100° for 9 hours, cooled, and 200 ml. of chloroform was added. The insoluble material (4.8 Gm.) was removed by filtration; the filtrate was washed successively with 50 ml. of water, two 50-ml. portions of saturated sodium bicarbonate solution, and two 50-ml. portions of water. The chloroform solution was dried over sodium sulfate; the solvent was removed on a steam bath giving 14.5 Gm. (40%) of a solid which was recrystallized from Skelly B; m.p. 65–66°. [Glick (3) reported m.p. 66° for the neutral product of this reaction. Synerholm and Hartzell (4) reported m.p. 66.5 to 68°.] The infrared spectrum (8% in chloroform) was identical

[1] All melting points are uncorrected. Elemental analyses were performed by Huffman Microanalytical Laboratories, Wheatridge, Colo.

in all respects with that of the product from *A* (8% in chloroform). Also, a mixed melting point determination with the product from *A* showed no depression.

Bis-(2-N,N-dimethylaminoethyl) Maleate.—This compound was prepared in 4% yield by the method of Christiansen and co-workers (6); b.p. 109–110° (0.1 mm.); literature (6) b.p. 145° (1.5 mm.).

Bis-(2-N,N-dimethylaminoethyl) Fumarate.—A 6.0-Gm. (0.018 mole) quantity of bis-(2-bromoethyl) fumarate and 3.6 Gm. (0.08 mole) of dimethylamine were added to 100 ml. of sodium-dried toluene in a 125-ml. Erlenmeyer flask fitted with a ground-glass stopper. The flask was stoppered and allowed to stand at room temperature for 1 month. The insoluble material was removed by filtration, the toluene was removed from the filtrate under reduced pressure, and the residue was distilled, whereupon 4.3 Gm. (90%) of material boiling at 105–106° (0.02 mm.) was collected; literature (16) b.p. 138° (2 mm.).

Dicholine Maleate and Dicholine Fumarate.—These compounds were prepared by the procedure described by Christiansen and co-workers (6) for the preparation of methiodides. Reagent grade acetone was employed; it was stored over anhydrous potassium carbonate. The methyl iodide was Eastman white label grade, and it was redistilled immediately before use. Both dicholine ester diiodides were recrystallized from methanol-water (5:1). Melting point determinations were performed in an open capillary tube which was placed in a rapidly stirred bath preheated to 230°, and the temperature was increased at the rate of 2° per minute. The melting points of the two esters were determined simultaneously, and they were both 252–253° with decomposition. A mixture of equal parts of the two esters melted at 252–253° with decomposition under conditions described above. Bovet and co-workers (16) reported 253° for dicholine fumarate and Christiansen and co-workers (6) reported 264° for dicholine maleate.

Pharmacology

Cats of each sex were anesthetized with α-chloralose and urethan (30 mg./Kg. in a 25% urethan solution). The dose utilized was calculated on α-chloralose as 55 mg./Kg.

After cannulating the trachea, the ipsilateral femoral nerve was severed. The posterior tibial nerve was separated from the peroneal nerve for a distance of 2 or 3 cm. The tibial nerve was ligated at a distance of 4 to 5 cm. from its union with the peroneal nerve and cut distal to the ligature. If bleeding occurred from the small vessels in the neural sheath, a piece of surgical gauze soaked in thrombin (100 NIH units/ml.) was applied to the nerve to aid in coagulation. The bony tubercle on the medial edge of the foot between the heel and toe was detached with bone clippers and the tendon of the tibialis anticus muscle ligated just above the tubercle with a strong thread. The distal end of the thread was attached to a muscle pulley lever system. The recordings of the contractions of the tibialis anticus muscle were made on a slowly moving smoked kymograph. Platinum tipped electrodes were fixed around the intact peroneal nerve. The nerve was bathed in warm heavy mineral oil maintained at 35 to 38°. A Grass stimulator (SD5) was used to deliver 0.8 to 1.0 volt square wave impulses every 6 seconds. The threshold for the peroneal nerve was determined for each preparation and control contractions recorded for a 30-minute period. A carotid artery was cannulated, and the blood pressure was recorded by means of a glass cannula and mercury manometer system. The glass cannula was connected to its respective pressure recording device through a normal saline (0.1% sodium chloride solution) bridge. Coagulation was prevented by injecting 0.2 ml. of heparin (1000 U.S.P. units/ml.) into the tip of the glass cannula. The experimental compound was administered *via* the femoral vein and the effects on contraction of the tibialis anticus muscle and blood pressure recorded. Succinyl dicholine was administered to each preparation.

REFERENCES

(1) Cavallito, C. J., and Gray, A. P., *Progr. Drug Res.*, **2**, 194(1960).
(2) Brücke, F., *Pharmacol. Rev.*, **8**, 271(1956).
(3) Glick, D., *J. Biol. Chem.*, **137**, 357(1941).
(4) Synerholm, M. E., and Hartzell, A., *Contrib. Boyce Thompson Insl.*, **14**, 79(1945).
(5) Fieser, L. F., and Fieser, M., "Advanced Organic Chemistry," Reinhold Corp., New York, N. Y., 1961, p. 95.
(6) Christiansen, J., Hansen, R. G., and Fakstorp, J., *Acta Chem. Scand.*, **1**, 128(1953).
(7) Mayo, F. R., and Walling, C., *Chem. Rev.*, **27**, 403 (1940).
(8) "The Merck Index," 6th ed., Merck and Co., Rahway, N. J., 1952, p. 596.
(9) Stafford, R. W., Shay, J. F., and Francel, R. J., *Anal. Chem.*, **26**, 656(1954).
(10) Knight, S., Bost, R. W., Shealy, O., and Williams J. P., *J. Am. Chem. Soc.*, **73**, 29(1951).
(11) Goldenberg, V., and Spoerri, P. E., *Anal. Chem.*, **31**, 1735(1959).
(12) Brante, G., *Nature*, **163**, 651(1949).
(13) Whittaker, V. P., and Wijesundera, S., *Biochem. J.*, **51**, 348(1952).
(14) Schilling, M., and Pedersen, J. G. A., *Arch. Exptl. Pathol. Pharmakol.*, **228**, 371(1956).
(15) Kyrides, L. P., "*Organic Synthesis*," Coll. Vol. **3**, John Wiley & Sons, New York, N. Y., 1953, p. 422.
(16) Fusco, R., Palazzo, G., Chiavarelli, S., and Bovet, D., *Gazz. Chim. Ital.*, **79**, 129(1949).