Emulsifying Effects of Several Ionic Surfactants on a Nonaqueous Immiscible System

By J. D. McMAHON, R. D. HAMILL, and R. V. PETERSEN

The emulsifying effects of twelve anionic and five cationic surfactants on the nonaqueous immiscible system, glycerin and olive oil, were studied. Three anionic agents, dioctyl sodium sulfosuccinate, diamyl sodium sulfosuccinate, and calcium stearate, and two cationic agents, benzalkonium chloride and lauryltrimethylammonium bromide, failed to stabilize the system. On the other hand, four anionic agents, tetrasodium-N-(1,2-dicarboxyethyl)-N-octadecyl-sulfosuccinamate, sodium lauryl ether sulfate, sodium lauryl sulfate, and sodium stearate, and three cationic agents, stearyltrimethylammonium chloride, cetylpyridinium chloride, and stearyldimethylbenzyl-ammonium chloride, produced stable emulsions. The anionic agents formed by the saponification of the fatty acids present in the olive oil with 2-amino-2-methyl-1,3-propanediol, tris (hydroxymethyl) aminomethane, triethanolamine, ethanolamine, or ammonia, yielded clear, stable emulsions. Emulsification occurred more readily, or preferentially, when the surfactant was first added to the glycerin. All of the emulsions formed were of the oil-in-glycerin type, except those containing sodium stearate or stearyltrimethylammonium chloride.

DURING RECENT YEARS rapid advances have been made in the technology of surface-active agents (surfactants). A multitude of new agents have been developed and their properties and actions studied. These studies have resulted not only in a vast expansion of the knowledge of the better known actions, but also in the discovery of new actions and new applications for these agents.

Surfactants exhibit many properties, among which are included emulsifying, demulsifying, wetting, foaming, antifoaming, dispersing, solubilizing, detergent, and spreading actions. Many of these actions are interrelated and all are manifestations of the thermodynamic function of surfactants in effecting a reduction in the surface or interfacial tension between a liquid and an adjoining phase. The amphiphilic nature of surfactant molecules results in a simultaneous attraction for both polar and nonpolar substances which accounts for some of their actions.

The actions enumerated above find applications in a large number of industries, including the pharmaceutical and cosmetic industries. Many pharmaceutical applications have been extensively investigated. For example, the use of surfactants as emulsifying agents for oil-water systems, as detergents, dispersing agents, suppository bases, disinfectants, preservatives, and fecal softeners, is well established. There are also reports in the literature regarding the use of surfactants as solubilizing agents for volatile oils (1), phenobarbital in a nonalcoholic vehicle (2),

reserpine (3), benzoic acid derivatives (4), hexachlorophene (5, 6), and many other substances for internal and external application. Many studies also have been performed and reviewed on the effects of surfactants on gastrointestinal absorption of drugs (7–9), absorption of fats (7), and percutaneous absorption (10). Despite the abundant literature regarding the effects of surfactants on aqueous and aqueous-oil systems, relatively little is mentioned on nonaqueous, binary, immiscible systems, such as glycerin and olive oil. For this reason, and because these solvents are pharmaceutically useful, these agents were selected as a model for the study of the effects of surfactants on such a system. The results obtained in the descriptive phase of this investigation provide the basis for this report.

METHODS

The system employed in each experiment consisted of equal weights of glycerin and olive oil with a variable concentration of surfactant. The glycerin and olive oil used were U.S.P. grade. Every reasonable precaution was taken to avoid undue exposure of these reagents to the atmosphere, except during actual manipulation; although, due to the hygroscopicity of glycerin, anhydrous conditions would have been almost impossible to achieve and maintain under the experimental conditions employed.

Two methods of mixing were used. In Method I the surfactant was weighed and added directly to a weighed quantity of glycerin. This mixture was then transferred to a glass mortar. The oil was added slowly in small portions, with rapid trituration after each addition. In Method II the requisite quantity of surfactant was added to the oil. The oil and surfactant were then transferred to a glass mortar and the glycerin was added slowly, in small portions, with vigorous trituration after each addition.

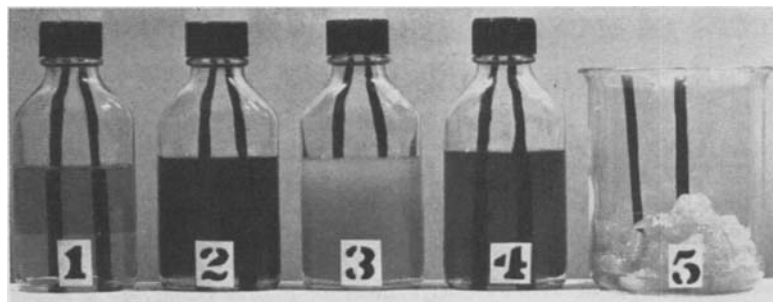Liquid surfactants were weighed directly into a tared beaker which contained the requisite weight

Fig. 1.—*1*, A typical separation; *2*, a partial separation; *3*, a somewhat opaque emulsion containing 3% Aerosol 22; *4*, a clear emulsion containing 1% triethanolamine; *5*, a semisolid emulsion containing 10% sodium lauryl sulfate.

of glycerin (Method I) or olive oil (Method II). Insoluble surfactants were incorporated into the glycerin or oil by warming the mixture on a water bath until solution or intimate mixing was effected.

The warm mixtures were allowed to cool to room temperature before being used.

In some cases it was advantageous to make stock solutions of surfactants in glycerin or oil. For

TABLE I.—EMULSIFYING EFFECT OF ANIONIC SURFACTANTS ON EQUAL WEIGHTS OF GLYCERIN AND OLIVE OIL

Agent	%	Method I[a]	Method II[a]	Emulsion, Appearance
Tetrasodium-N-(1,2-dicarboxy-ethyl)-N-octadecyl-sulfosuc-cinamate[b]	1.0	Emulsification	...	Opaque
	3.0	Emulsification	Separation	Opaque
	5.0	Emulsification	...	Opaque
Dioctyl sodium sulfosuccinate[c]	1.0	Separation[d]	Separation[d]	...
	3.0	Separation[d]	Separation[d]	...
Diamyl sodium sulfosuccinate[e]	1.0	Separation[d]	Separation[d]	...
	3.0	Separation[d]	Separation[d]	...
Sodium lauryl ether sulfate[f]	2.0	Emulsification	...	Opaque
	5.0	Emulsification	Separation	Opaque
Sodium lauryl sulfate	2.0	Partial sep.[d]	Separation	...
	5.0	Partial sep.[d]	Separation	...
	10.0	Emulsification	Separation	Opaque, semisolid
Sodium stearate	2.0	Emulsification[g]	Separation	Opaque, semisolid
Calcium stearate	2.0	Separation[g]	Separation[g]	...
	5.0	Separation[g]	Separation[g]	...
2-Amino-2-methyl-1,3-propane-diol[h]	0.05	Separation[d]	...	...
	0.1	Separation[d]	...	...
	0.15	Emulsification	...	Clear
	0.25	Emulsification	...	Clear
	0.5	Emulsification	Separation	Clear
	1.0	Emulsification	Separation	Clear
	2.0	Emulsification	Separation	Clear
Ammonia (gas)	0.01	Emulsification	...	Clear
	0.025	Emulsification	...	Clear
	0.05	Emulsification	...	Clear
	0.1	Emulsification	Separation	Clear
	0.15	...	Separation	...
	0.2	...	Emulsification	Clear
	0.25	Emulsification	Emulsification	Clear
	0.3	...	Emulsification	Clear
	0.5	Emulsification	...	Clear
	1.0	Emulsification	...	Clear
Ethanolamine	2.0	Partial sep.[d]	Separation	...
	5.0	Partial sep.[d]	Separation	...
	10.0	Emulsification	Separation	Opaque, semisolid
Triethanolamine	0.063	Separation[d]	Separation	...
	0.125	Separation[d]	Separation	...
	0.25	Separation[d]	Separation	...
	0.5	Emulsification	Separation	Clear
	1.0	Emulsification	Separation	Clear
	2.0	Emulsification	Separation	Clear
	3.0	Emulsification	Separation	Clear
	4.0	Emulsification	Separation	Clear
	5.0	Emulsification	Separation	Clear
Tris (hydroxymethyl) amino-methane[h]	0.1	Emulsification	...	Clear
	0.25	Emulsification	...	Clear
	0.5	Emulsification	...	Clear
	1.0	Emulsification	Separation	Clear

[a] See text. [b] Marketed as Aerosol 22 by American Cyanamid Co., Pearl River, N. Y. [c] Marketed as Aerosol OT by American Cyanamid Co., Pearl River, N. Y. [d] Emulsification was apparent by macroscopic and microscopic examination, but separation was detectable within 7 days. [e] Marketed as Aerosol AY by American Cyanamid Co., Pearl River, N. Y. [f] Marketed as Sipon ES by American Alcolac Corp. [g] Surfactant not soluble; dispersion formed on heating. [h] Commercial Solvents Corp.

example, to expedite the use of ammonia as a saponifying agent, stock solutions were prepared by bubbling the anhydrous gas into weighed quantities of glycerin and of olive oil. Requisite quantities of these stock solutions were used for the preparation of solutions of various concentrations.

Hand methods of trituration were employed throughout the experiment. A colloid mill and a hand homogenizer were used to compare the results obtained by hand methods, but these devices did not improve significantly the products obtained by hand trituration.

Three criteria were used to evaluate the results of manipulations by Methods I and II. The product was considered to be *stable* if emulsification occurred and there was no separation into layers for at least 7 days. The term *emulsions* is used to designate those products which did not separate into distinct layers, although solubilization may have contributed to the overall phenomenon. Figure 1 depicts the appearance of a completely separated product, a partially separated product, a clear emulsion, an opaque emulsion and a semisolid emulsion, all of which were aged for at least 1 week. Only the latter three examples were considered as emulsions. Another criterion was based on the *miscibility* of the emulsion with added olive oil or glycerin upon gentle stirring. This procedure was employed to determine emulsion type, based on the assumption that additional liquid of the external phase, but not of the internal phase, could easily be mixed with the emulsion. Finally, *microscopic* determination of emulsion type was facilitated by the preparation of a 1.0% solution of D & C red No. 18 dye in olive oil. Heat was applied to effect solution. The cooled oil, containing the dye, was subsequently mixed with glycerin and surfactants in the usual manner and the resulting emulsions examined microscopically.

To determine the influence of temperature on the miscibility of olive oil and glycerin, mixtures of these agents were prepared in increments of 5.0% ranging from 5.0% olive oil and 95.0% glycerin to 95.0% olive oil and 5.0% glycerin. Miscibility was not effected in any of these mixtures at temperatures up to 180°, at which point there was evidence of decomposition.

RESULTS

Anionic Agents.—Table I summarizes the results obtained using the anionic surfactants and the saponifying amines.

Cationic Agents.—Table II summarizes the results obtained using the cationic agents.

An examination of Table I reveals that stable emulsions were obtained from the use of tetrasodium - N - (1,2 - dicarboxyethyl) - N - octadecylsulfosuccinamate, sodium lauryl ether sulfate, sodium lauryl sulfate, sodium stearate, and from the saponifying amines, 2-amino-2-methyl-1,3-propanediol, tris(hydroxymethyl) aminomethane, triethanolamine, ethanolamine, and ammonia. Other agents produced emulsions which remained stable for several hours or days, but separated partially or completely on standing. For example, emulsification was observed immediately after manipulation of products containing dioctyl sodium sulfosuccinate, diamyl sodium sulfosuccinate, the lower concentrations of 2-amino-2-methyl-1,3-propanediol, or triethanolamine. Negative results are reported, however, because detectable separation occurred within the arbitrary 7-day period.

Microscopic examination of the products containing D & C red No. 18 dye, predissolved in the oil, confirmed the fact that emulsification had occurred. Figures 2 and 3 illustrate this observation. In all cases, except that of sodium stearate, the oil appeared in the internal phase, although evidence of

TABLE II.—EMULSIFYING EFFECTS OF CATIONIC SURFACTANTS ON EQUAL WEIGHTS OF GLYCERIN AND OLIVE OIL

Agent	%	Method I[a]	Method II[a]	Emulsion, Appearance
Benzalkonium chloride[b]	0.1	Separation	Separation	...
	0.2	Separation	Separation	...
	0.5	Separation	Separation	...
	1.0	Separation	Separation	...
	2.0	Separation	Separation	...
	5.0	Separation	Separation	...
	10.0	Separation	Separation	...
Lauryltrimethylammonium bromide[c]	0.1	Separation	[e]	...
	0.5	Partial sep.[d]	...	...
	1.0	Partial sep.[d]	...	...
	5.0	Partial sep.[d]	...	...
Cetylpyridinium chloride[c]	0.1	Separation	Separation	...
	0.5	Emulsification	Separation	Opaque
	1.0	Emulsification	Separation	Opaque
	5.0	Emulsification	Separation	Opaque
Stearyltrimethylammonium chloride[c]	0.1	Separation	Separation	...
	0.5	Partial sep.	Partial sep.	...
	1.0	Emulsification	Partial sep.	Opaque
	5.0	Emulsification	Emulsification	Opaque
Stearyldimethylbenzylammonium chloride[c]	0.1	Separation	Separation	...
	0.5	Emulsification	Separation	Opaque[f]
	1.0	Emulsification	Separation	Opaque[f]
	5.0	Emulsification	Separation	Opaque[f]

[a] See text. [b] Benzalkonium chloride (Zephiran Chloride, Winthrop Laboratories) was obtained by evaporation of a 12.8% aqueous solution and as the 92.7% semisolid. Both were employed in these experiments. The 92.7% semisolid produced emulsions in the higher concentrations, but separation occurred within 7 days. [c] Obtained from K & K Laboratories, Inc. [d] Emulsification was apparent by macroscopic and microscopic examination, but separation was detectable within 7 days. [e] Insoluble in the olive oil. [f] Opacity due to precipitated surfactant.
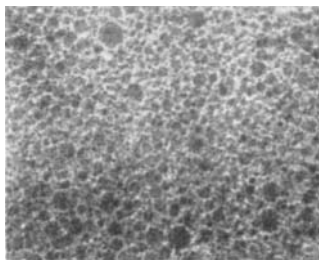
Fig. 2.—Photomicrograph of an emulsion containing 1.0% triethanolamine in equal weights of glycerin and olive oil stained with D & C red No. 18 dye.
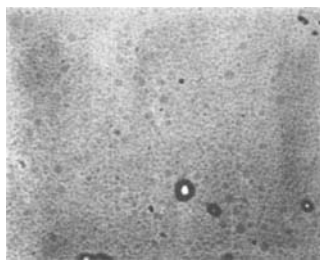

Fig. 3.—Photomicrograph of an emulsion containing 3% Aerosol in equal weights of glycerin and olive oil stained with D & C red No. 18 dye.

mixed emulsification was observed in a number of instances. Figure 4 is a photomicrograph of a sodium stearate emulsion wherein the oil (dark area) is seen in the external phase. Mixed emulsification can be detected by close observation of this figure.

When sodium lauryl sulfate, sodium stearate, or ethanolamine was used, the resulting preparations had a very thick consistency. Indeed, the higher concentrations resulted in semisolid preparations. Consequently, any separation in these products occurred very slowly, usually over several weeks.

With the exception of ethanolamine, the saponifying amines produced very stable emulsions, even in very low concentrations. The resulting products were clear or only faintly cloudy. Several of them exhibited opalescence. Some darkening was noted in emulsions containing triethanolamine or ammonia on standing. However, some of these preparations have remained stable for over 2 years. It is interesting that the use of preformed triethanolammonium oleate, prepared by reacting equivalent amounts of triethanolamine and oleic acid, aided by mild heating, produced the same results as did the corresponding amount of triethanolamine.

Table II reveals that stable emulsions were obtained from the use of the cationic agents, cetylpyridinium chloride, stearyltrimethylammonium chloride, and stearyldimethylbenzylammonium chloride. Emulsions were formed also when benzalkonium chloride or lauryltrimethylammonium bromide were used; however, these emulsions separated, partially or completely, within 7 days. That emulsification occurred in these preparations was confirmed by microscopic examination of the dyed emulsions.

An inversion in emulsion type was observed when stearyltrimethylammonium chloride was employed in concentrations of 0.5 and 1.0% by Method I. Preparations containing 0.5% of this agent formed oil-in-glycerin emulsions until the proportion of oil reached approximately 42% of the total composition, at which point an inversion to the glycerin-in-oil type occurred. Partial separation of this emulsion was detectable after standing for 7 days. Preparations containing 1.0% stearyltrimethylammonium chloride, prepared by Method I, formed oil-in-glycerin emulsions until the proportion of oil reached approximately 48%, at which point inversion occurred. No separation of these emulsions was detectable after 7 days. At the 5.0% level of surfactant inversion to the glycerin-in-oil type did not occur, even when the oil concentration was increased to approximately 78–80%. At this point the emulsions "cracked."

The use of stearyltrimethylammonium chloride by Method II produced emulsions of the glycerin-in-oil type at concentrations of 0.5, 1.0, and 5.0%,

although at the two lower concentrations partial separation occurred on standing for 7 days.

A comparison of the results obtained with Method I and Method II reveals that the former was superior in its ability to produce stable emulsions with either anionic or cationic surfactants. Method II was successful only in the case of ammonia, in one preparation containing 1.0% triethanolamine which had been aged for several days (not included in Table I), and in preparations containing stearyltrimethylammonium chloride.

DISCUSSION

The data presented in Table I indicate that certain anionic agents are useful for emulsifying the glycerinolive oil system employed in these studies. This interpretation is supported by macroscopic and microscopic examination and by testing the products for miscibility with added olive oil or glycerin. Except for sodium stearate emulsions, all of the emulsions prepared from anionic agents were of the oil-in-glycerin type. Emulsions containing sodium stearate were of the glycerin-in-oil type. The sodium stearate–induced emulsions exhibited a degree of mixed emulsification, as did a number of other emulsions. This can be observed in Fig. 4. The glycerin-in-oil nature of emulsions containing sodium stearate was further substantiated by the fact that only a limited quantity of glycerin could be added to these emulsions, whereas olive oil was readily miscible with them. In all other instances glycerin, but not olive oil, mixed readily with the emulsions.

Emulsions obtained with 2-amino-2-methyl-1,3-propanediol, tris(hydroxymethyl) aminomethane, triethanolamine, ethanolamine, or ammonia, using Method I, formed rapidly with a minimum of agitation. Evidently, saponification occurred readily. The great capacity of these agents to emulsify the oil is remarkable, since, in one case, as little as 0.01% of the saponifying amine was used to produce a stable emulsion. Still lower concentrations may be effective for this purpose.

The value of the cationic surfactants as emulsifying and solubilizing agents has not been unequivocally established. The solubilizing effects of dodecylamine hydrochloride (11) and cetylpyridinium chloride (12) in aqueous preparations have been reported. The results presented herein indicate that cetylpyridinium chloride, stearyltrimethylammonium chloride, and stearyltrimethylbenzylammonium chloride are effective agents for the emulsification of olive oil and glycerin under the conditions of this experiment and may be of value in this application. These observations should be extended before categorical statements can be made regarding
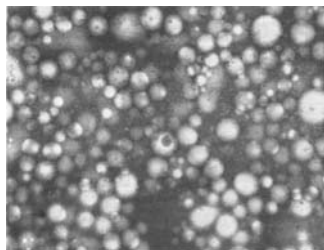
Fig. 4.—Photomicrograph of an emulsion containing 2.0% sodium stearate in equal weights of glycerin and olive oil stained with D & C red No. 18 dye.

the usefulness of these agents in solubilizing or emulsifying aqueous or nonaqueous systems.

The results reported in Table II on the cationic agents were substantiated by microscopic and macroscopic examination and by testing the products for stability and for miscibility with added olive oil or glycerin.

The fact that both types of emulsions, oil-in-glycerin and glycerin-in-oil, were induced by one agent, stearyltrimethylammonium chloride, lends support to the well known theories which imply that an emulsion forms in such a way that the higher proportion of surfactant is in the external phase. Indeed, with this agent, Method I (in which the surfactant was first dissolved in glycerin) favored the formation of oil-in-glycerin emulsification, whereas the reverse was true of Method II. The inversions observed during manipulation by Method I did not occur until substantial quantities of oil had been incorporated into the internal phase.

The viscosity of emulsions resulting from the use of either anionic or cationic agents was obviously much higher than that of either of the constituents, although accurate viscosity measurements have not yet been made. Even when emulsification was not effected, an increase in viscosity and slower rates of separation, compared to olive oil and glycerin alone, frequently were noted.

It was observed that in many instances where negative results are indicated, emulsification resulted until the proportion of oil exceeded a maximum concentration at which point separation occurred. This point was frequently very sharp, occurring on addition of only 1 or 2 ml. of oil and detectable by a sudden decrease in viscosity. This phenomenon was particularly noticeable with the lower concentrations of saponifying amines. For such cases it is assumed that the concentration in internal phase exceeded the capacity of the surfactant to maintain a stable emulsion. It is realized that greater variation in the concentrations of surfactants or in the proportion of glycerin and/or olive oil may have produced positive results where emulsification otherwise did not occur.

The addition of an oil soluble dye (D & C red No. 18) or a glycerin soluble dye (methylene blue) to the *finished emulsion* was unsatisfactory for microscopic observation and led to some early erroneous conclusions. The fact that most of the nondyed emulsions were translucent and failed to show emulsification on microscopic examination led to the initial belief that solubilization was the primary action of the surfactants in this system. This is understandable in view of the similar refractive indices of the reagents (glycerin n_D^{25} 1.4730; olive oil n_D^{25} 1.466–1.468). This also may account for the fact that

most of the emulsions were clear or only faintly cloudy.

Technique played an important role in the successful formation of emulsions. Vigorous trituration and slow addition of the oil enhanced the chances of effecting emulsification. Microscopic examination of the dyed emulsions revealed that those prepared by this technique exhibited smaller globule sizes than those prepared by less vigorous agitation and more rapid addition of the oil. It should be mentioned, however, that the surfactant also had a pronounced influence on the size of the globules contained in the emulsion.

Why is Method I superior to Method II in its capacity to produce stable emulsions? The answer to this question is not obvious. However, based on the assumption that micelle formation is responsible for eventual emulsification, an explanation might be proposed. McBain (13) has suggested that emulsification results when the saturation point of micelles is exceeded and further states: "The apparent density of liquid solubilized by a micelle is the same or nearly the same as that of the pure oil at the limit of saturation (as may easily be seen on reflecting that when the micelle becomes saturated the addition of still more liquid results in a *transition from solution to emulsion*)." The existence of micelles in nonaqueous systems has been reported by several investigators (14–20). Hall (21), on the other hand, has evidence that micelles are not present in benzene solutions of the nonionic agent, polyoxyethylene lauryl ether 30,[1] but has evidence supporting the existence of micelles in benzene solutions of anionic and cationic surfactants. The fact that glycerin is a polar substance and has solvent properties quite similar to those of water would lead one to suspect that micelles form in glycerin in a manner similar to the micelle formation in water. The assumption that micelles do exist and that the oil is dissolved within the hydrocarbon interior of the micelle appears logical. The fact that emulsions formed quickly and easily by use of the saponifying amines indicates that micellar formation was rapid. It would further appear that once a micelle forms, the orientation is highly stable and a reversion in type does not readily occur. Thus, the micellar orientation within the system employed in these studies which leads to stable emulsions must, in most instances, be that present in glycerin.

SUMMARY

The effects of various surfactants on the system, olive oil and glycerin, were studied. The anionic agents, dioctyl sodium sulfosuccinate, diamyl sodium sulfosuccinate, and calcium stearate failed to produce stable emulsions under the conditions of this study. The cationic agents, benzalkonium chloride and lauryltrimethylammonium bromide, likewise failed to stabilize the system.

Some anionic agents were effective in producing emulsification of glycerin, and olive oil. Tetrasodium - N - (1,2 - dicarboxyethyl) - N - octadecyl-sulfosuccinamate, sodium lauryl ether sulfate, and sodium lauryl sulfate produced stable, somewhat opaque, preparations. The amines, 2-amino-2-methyl-1,3-propanediol, tris(hydroxymethyl) amino-

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.

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Comparative Activities of Maleyl, Fumaryl, and Succinyl Dicholine

A Correction of the Literature

By JOHN F. McCARTHY, JOSEPH G. CANNON†, and JOSEPH P. BUCKLEY

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, respec-

tively, 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;[1] 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