Identification and Differentiation of Organic Medicinal Agents V

Quaternary Ammonium Compounds

By LESLIE G. CHATTEN, ANN C. NAPPER, and PAMELA JANE BARRY

Identification tests have been presented for 23 compounds containing either one or two quaternary ammonium functional groups. The reactions of these compounds with ammonium reineckate, picric acid, and chloroplatinic acid have been studied and 54 adducts have been isolated and their melting ranges presented. In addition, microcrystal tests showed that many adducts possessed distinctive crystalline morphology. Photomicrographs are presented as a supplemental and additional parameter for characterization purposes.

MEDICINAL AGENTS containing a quaternary ammonium function comprise several pharmacologically heterogeneous groups such as cationic surface-active agents, parasympathomimetics, parasympatholytics, skeletal muscle relaxants, or antagonists of curariform drugs, just to mention a few. Despite the broad scope and importance of these compounds, satisfactory methods for their identification are not available. Although a number of the compounds investigated in this report have official status in either the B.P. (1), the U.S.P. (2), or the N.F. (3), in general, the identification tests employed in these compendia are nonspecific.

It is the purpose of this investigation to develop identification tests of greater specificity, in order to permit differentiation between the various quaternary ammonium compounds.

EXPERIMENTAL

Apparatus—Thomas-Hoover melting point apparatus (calibrated with melting point reference standards); Leitz Laborlux-Pol. microscope equipped with a Zeiss Ikon 35 mm. camera.

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Quaternary Ammonium Compounds—Drugs investigated are listed in Table I.

Reagents and Solutions—All reagents used were A.C.S. grade, or reagent grade if the former was not commercially available. Ammonium reineckate 1% aqueous solution; picric acid saturated ethanolic solution; chloroplatinic acid 10% in diluted HCl; solutions of quaternary ammonium compounds, ammonium reineckate, picric acid, and chloroplatinic acid for microcrystallography are listed in Table II.

Formation of Derivatives

All derivatives were dried *in vacuo* over phosphorus pentoxide for 24 hr. prior to determining their respective melting points on a Thomas-Hoover melting point apparatus. All melting points were corrected by reference to a calibration curve.

Reineckates—The reineckates were prepared according to the following modified procedure of Chatten and Levi (4). With the exception of isopropamide iodide, mepenzolate bromide, and dithiazinine iodide, 1 mmole of quaternary ammonium salt was dissolved in a minimum amount of water. While stirring vigorously, 1 mmole of ammonium reineckate in aqueous solution was added for each quaternary ammonium functional group present in the parent compound. Isopropamide iodide and mepenzolate bromide were dissolved in a minimum of ethanol, while acetone was selected as the most satisfactory solvent for dithiazinine iodide.

After cooling for 2 hr. or until precipitation was complete, the reineckate crystals were filtered, dried, and then recrystallized by dissolving in a minimum of acetone and adding water to the cloud point. The solution was cooled until recrystallization was complete. For certain reineckates, *i.e.*, those of cetylpyridinium, pyridostigmine, edrophonium, propantheline, methantheline, chlorisondamine, demecarium, and domiphen, compounds of high purity could only be obtained by washing them with ethanol prior to drying.

Elemental analyses (C, H, N) confirmed the purity and identity of the reineckates.

Picrates—To 1 mmole of the quaternary ammonium salt, dissolved in water, 1 mmole of picric acid in 10 ml. of ethanol was added for each quaternary ammonium functional group. The mixture was allowed to stand in the refrigerator for 30 min. and the crystals were removed by suction filtration. Recrystallization was accomplished by dissolving the

TABLE I—MELTING POINTS OF DERIVATIVES OF QUATERNARY AMMONIUM COMPOUNDS

Parent Compd.	Reineckates, °C.	Picrates, °C.	Chloroplatinates, ° C.
Ambenonium chloride	168–169	205 dec.	206–207
Benzethonium chloride	158–160	Oil	165.5–167
Bethanechol chloride	160 dec.	126–130	194.5–196.5
Cetylpyridinium chloride	131–133	50–50.5	187–188
Chlorisondamine chloride	183–188	246–247 dec.	Carbon analysis not theoretical
Demecarium bromide	180–185	Oil	...
Dithiazinine iodide	...	220	...
Domiphen bromide	126–127.5	Oil	197–198
Echothiophate iodide	129–140 dec.	Oil	Black mass
Edrophonium bromide	...	161–161.5	194–194.5 dec.
Hexamethonium chloride	272–275 dec.	239.5–241 240 (1)	282–293 dec.
Hexocyclium methylsulfate	191–192	189–190	224–226 dec.
Isopropamide iodide	154–155	140–140.5	Black mass
Mepenzolate bromide	166–167	160–161	Carbon analysis not theoretical
Methantheline bromide	131.5–135	114.5–115	187–188.5
Oxyphenonium bromide	130–132	101.5–102	198–200 dec.
Penthienate bromide	142–146	Oil	149
Pentolinium tartrate	272–276 dec.	270–271 dec. 270 (1)	233.5–234.5 dec.
Pipenzolate methylbromide	158–159 dec.	160.5–162	193–196
Propantheline bromide	134–137	Oil	165–167
Pyridostigmine bromide	140–143 153–156 (2)	115–115.5	160–162
Trimethidinium methosulfate	250–251	300–301	255
Valethamate bromide	128–130	Oil	174.5–177.5

TABLE II—QUATERNARY AMMONIUM COMPOUNDS CHARACTERIZED BY MICROCRYSTALLOGRAPHY[a]

Parent Compd.	Ammonium Reineckate Parent:Reineckate	Picric Acid Parent:Picric Acid	Chloroplatinic acid Parent:Chloroplatinic Acid
Ambenonium chloride	Oil globules	0.125:0.125	0.125:0.25
Benzethonium chloride	Oil globules	Oil globules	Brownish mass
Bethanechol chloride	4:0.3	Oil globules	...
Cetylpyridinium chloride	0.5:0.025	Oil globules	Brownish mass
Chlorisondamine chloride	1:1	0.062:0.125	Oil globules
Demecarium bromide	Oil globules	Oil globules	Oil globules
Dithiazinine iodide	Dithiazinine crystals	Dithiazinine crystals	Dithiazinine crystals
Domiphen bromide	1:1	Oil globules	Oil globules
Echothiophate iodide	2:0.1	Oil globules	Black mass
Edrophonium bromide	10:1	1.0:0.5	1.0:1.0
Hexamethonium chloride	0.007:0.025	0.5:0.5	0.5:0.5
Hexocyclium methylsulfate	1:1	Oil globules	Oil globules
Isopropamide iodide	Oil globules	Oil globules	Oil globules
Mepenzolate bromide	1:0.25	Oil globules	0.25[b]:0.125
Methantheline bromide	Oil globules	Oil globules	Brownish mass
Oxyphenonium bromide	5:0.1	Oil globules	Amorphous
Penthienate bromide	0.015:0.3	Oil globules	Oil globules
Pentolinium tartrate	0.5:1	0.5:0.5	0.5:0.5
Pipenzolate methylbromide	0.25:0.15	Oil globules	Oil globules
Propantheline bromide	5:0.1	Oil globules	Brownish mass
Pyridostigmine bromide	2:0.5	2.0:2.0[b]	4.0:1.0
Trimethidinium methosulfate	2:0.5	0.25:0.125	0.25:0.125
Valethamate bromide	0.05:0.3	Oil globules	Amorphous

[a] Concentrations of solutions are expressed in percentages. Time when picture was taken: 1–5 min. [b] Ethanol (95%) solution.

derivative in a minimum of ethanol or acetone and adding water slowly until the cloud point was reached. The mixture was set aside in the refrigerator to recrystallize. Elemental analyses (C, H, N) verified the purity and identity of the picrates. For the three quaternary ammonium parent compounds which are insoluble in water, solvents utilized are outlined under *Reineckates*.

Chloroplatinates—To 1 mmole of the quaternary ammonium salt, dissolved in a minimum of water, 1 mmole of chloroplatinic acid in dilute HCl was added for each quaternary ammonium functional group in the molecule. The resulting mixture was cooled in an ice bath for 20 min. or overnight in the refrigerator. The fine precipitate was filtered off, washed with cold water, and recrystallized from

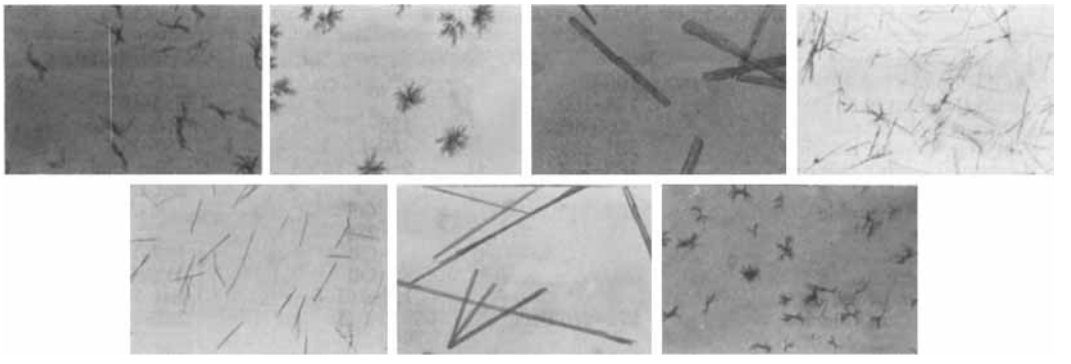

Fig. 1—Photomicrographs of the picrates. Top: ambenonium, chlorisondamine, edrophonium, hexamethonium. Bottom: pentolinium, pyridostigmine, trimethidinium. (Left to right.)

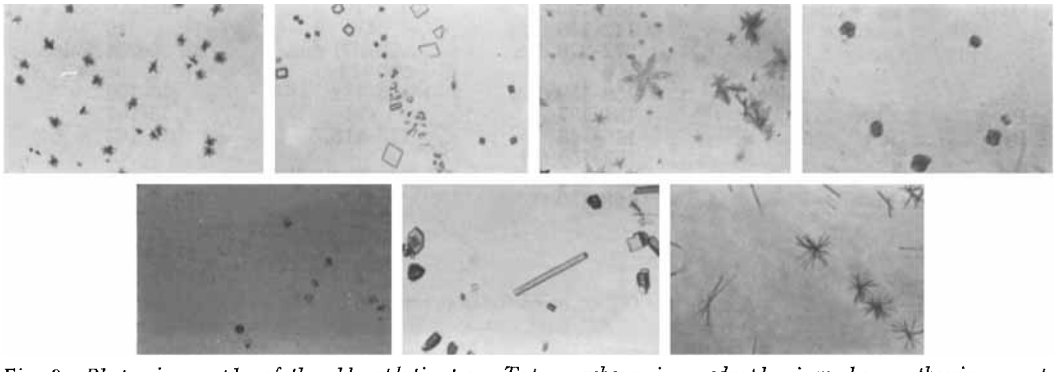

Fig. 2—Photomicrographs of the chloroplatinates. Top: ambenonium, edrophonium, hexamethonium, mepenzolate. Bottom: pentolinium, pyridostigmine, trimethidinium. (Left to right.)

boiling ethanol. If the adduct was insoluble in hot ethanol, it was washed well with cold methanol. In those three instances where the quaternary ammonium parent compound was not soluble in water, they were dissolved in ethanol and the chloroplatinic acid was added as an aqueous methanolic solution.

Elemental analyses (C, H, N) confirmed the purity and identity of the chloroplatinates.

Photomicrographs

To 1 drop of an aqueous or alcoholic solution of each quaternary ammonium salt placed on a microscope slide, 1 drop of reagent solution (ammonium reineckate, picric acid, or chloroplatinic acid) was added. The solutions were mixed well and covered with a cover glass. One to five minutes was allowed for the formation of crystals, and the pictures were taken at a magnification of approximately 100×.

RESULTS AND DISCUSSION

Examination of the official compendia (1-3) reveals that 13 of the 23 quaternary ammonium compounds are official in one or more compendia. In only three monographs are derivatives used as a means of identification. Two are picrates and one a reineckate. Monographs of the other compounds rely, to a large extent, upon identification by a series of relatively nonspecific color and precipitation tests. All adducts prepared in this investigation are presented in Table I, together with their melting ranges and previously reported literature values.

Reineckates—In general, ammonium reineckate proved to be a very useful reagent for the preparation of derivatives, although products of satisfactory purity could not be obtained for either dithiazinine or edrophonium. For cetylpyridinium, chlorisondamine, demecarium, methantheline, propantheline, and pyridostigmine repeated crystallization failed to yield products which gave acceptable elemental analysis. However, adducts of high purity were obtained after these materials were well washed with ethanol prior to drying. According to the official compendia (1, 2) pyridostigmine reineckate melts at 153–156°. The results of this investigation revealed that only that derivative which melted at 140–143° gave satisfactory elemental analysis.

Examination of Table I reveals that a certain amount of overlap of melting points exists between several derivatives. Therefore, reineckates cannot be used as the sole criterion for identification purposes.

Picrates—Sixteen of the 23 quaternary ammonium compounds gave adducts of high purity with picric acid. For each of the remaining seven compounds, a viscous oil was obtained which failed to crystallize. Examination of Table I reveals that the picrates are highly satisfactory derivatives in that a minimum of overlapping of melting ranges occurs between compounds.

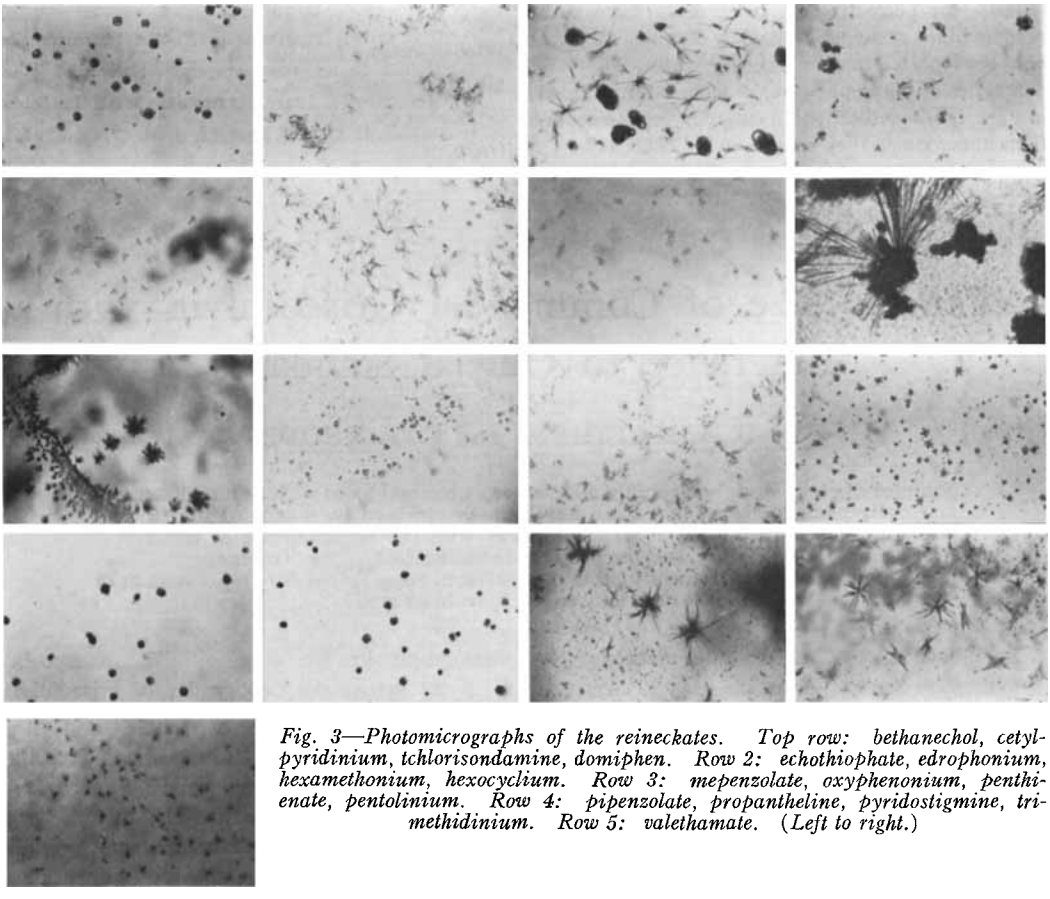

Fig. 3—Photomicrographs of the reineckates. Top row: bethanechol, cetylpyridinium, tchlorisondamine, domiphen. Row 2: echothiophate, edrophonium, hexamethonium, hexocyclium. Row 3: mepenzolate, oxyphenonium, penthienate, pentolinium. Row 4: pipenzolate, propantheline, pyridostigmine, trimethidinium. Row 5: valethamate. (Left to right.)

Chloroplatinates—Although some overlapping of melting ranges occurs in a few instances, the 17 derivatives that resulted with this reagent are valuable aids in the identification of the corresponding parent compound.

Attempted preparation of the chloroplatinate from isopropamide iodide and echothiophate iodide resulted immediately in a black decomposed mass from which no satisfactory product could be isolated. Demecarium bromide and dithiazinine iodide gave impure products which could not be purified by recrystallization. Chlorisondamine and mepenzolate chloroplatinates were crystalline compounds in which the carbon analysis did not correspond with theoretical values, even after repeated crystallizations.

Photomicrography

The three reagents employed in the preparation of the derivatives were utilized in the characterization studies by microcrystallography. Table II, a compilation of the experimental results, shows the percentage concentration of quaternary ammonium compound to that of reagent when crystals occurred. Other results such as oil globules or amorphous mass are recorded in the appropriate columns. In some instances, each of the three reagents produced crystals with a particular quaternary ammonium compound. Generally, success was noted with at least one of the reagents employed. However, exceptions to this were benzethonium chloride, demecarium iodide, isopropamide iodide, methantheline bromide, oxyphenonium bromide, and dithiazinine iodide. With the last named material, crystals of the parent compound appeared repeatedly.

Figure 1 shows the photomicrographs of those compounds which gave crystalline picrates, while Fig. 2 illustrates the chloroplatinates. While each of these reagents was useful in only a limited number of instances, the crystalline morphology of the derivatives depicted is remarkable. Figure 3 presents the photomicrographs of the 17 reineckates and most of these offer unique crystalline habits. Thus the photomicrographs of these three figures would be of high utility in differentiating the parent compounds. Like other physicochemical criteria, however, one should not rely upon one single criterion such as crystalline morphology for identification. It is an aid to identification just as color tests or derivatives are.

It is interesting to note the crystals of ambenonium reineckate and isopropamide reineckate were obtained once, but since neither could be repeated, their photographs were not included in Fig. 3.

SUMMARY

A series of physical chemical criteria, by which 23 quaternary ammonium compounds can be identified and differentiated, has been presented.

Fifty-four derivatives of these drugs have been prepared in a systematic manner, of which 52 have not been reported to date in the literature.

A series of photomicrographs have been included in Figs. 1–3, together with a summary of the conditions under which they were formed (Table II).

REFERENCES

(1) "The British Pharmacopoeia," The Pharmaceutical Press, London, England, 1963.
(2) "United States Pharmacopeia," 17th rev., Mack Publishing Co., Easton, Pa., 1965.
(3) "National Formulary," 12th ed., Mack Publishing Co., Easton, Pa., 1965.
(4) Chatten, L. G., and Levi, L., *Anal. Chem.*, **31**, 1581 (1959).

Particle Size of Commercial Griseofulvin with Reference to Official Standards

By B. A. MATTHEWS and C. T. RHODES

Samples of commercial griseofulvin have been obtained from various manufacturers and their particle size distribution has been determined by means of the Coulter counter. These results have been compared with the requirements of the monographs in the B.P., U.S.P., and the proposed specification of the "International Pharmacopoeia" and, in the light of this comparison, suggestions for improvement of these specifications have been made.

GRISEOFULVIN (7-chloro-4,6-dimethoxycoumaran - 3 - one - 2 - spiro - 1′ - [2′ - methoxy-6′-methylcyclohex-2′-en-4′-one]) has gained widespread acceptance in the oral treatment of fungal infections in man (1). It is the subject of monographs in a number of pharmacopeias (2–6). Early work on serum and tissue levels of the drug in rats, by Bedford *et al.* (7), and in humans, by McNall (8), suggested that the drug is poorly absorbed from the gastrointestinal tract. Further confirmation of this was published by Sharpe and Tomich (9), who were unable to determine the lethal dose of orally administered griseofulvin in rats and mice and by Gonzales-Ochoa *et al.* (10), who attempted to treat dermatophytoses in man.

All drugs, whether readily or sparingly soluble, dissolve more rapidly when finely divided, and Nelson (11, 12) showed that for theophylline salts and tetracycline, the rate of appearance of the drug in the blood stream following oral administration was determined by the solution rate. Griseofulvin is almost insoluble in water (13) and several attempts have been made to determine the effect of particle size on blood levels in animals (14) and man (15–17). Kraml *et al.* (17) found that a 0.5-Gm. dose of micronized griseofulvin produced serum levels indistinguishable from those produced by a 1.0-Gm. dose of nonmicronized griseofulvin. The monograph in the "British Pharmacopoeia" 1963 (2) recognizes two grades of griseofulvin called, respectively, "coarse particle" and "fine particle," whereas the "United States Pharmacopeia" (4) specifies one called "microsize," which corresponds approximately to the "fine particle" of the "British Pharmacopoeia." In each case some particle size requirements are given. The Food and Drug Administration monograph on microsize griseofulvin specifies additional particle size requirements based on specific surface area measurements by an air permeability technique. The limits are 1.3 to 1.6 sq. M./Gm. The proposed monograph for griseofulvin in the Volume of Specifications of the second edition of the "International Pharmacopoeia," to be published later this year, has a particle size specification based on a sieve technique. A limit of 5% by weight is allowed to be retained on a 300-mesh sieve (aperture size 53 μ).

The authors have obtained samples of commercial griseofulvin from several sources and by means of the Coulter counter (18, 19) have examined these to determine their particle size distribution. They have also utilized microscopy and specific surface area measurements to check results and have compared these with the particle size requirements of the pharmacopeias.

EXPERIMENTAL

Apparatus—A Coulter counter model B[1] with 50-μ and 140-μ orifices was used to determine the particle size. The electrolyte was 1% sodium

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[1] Marketed by Coulter Electronics Ltd., Dunstable, Bedfordshire, England.