Identification and Differentiation of Organic Medicinal Agents III

Amine-Containing Antiparkinson Agents and Newer Muscle Relaxants

By LESLIE G. CHATTEN and LYNN A. DOAN*

A series of specific physical criteria, by which four amine-containing antiparkinson agents and a newer amine-containing skeletal muscle relaxant, currently in popular clinical application, can be identified and differentiated, is presented. Twenty-three derivatives of these compounds were prepared using a variety of characterizing agents which included picric acid, ammonium reineckate, sodium tetraphenylborate, chloroplatinic acid, and methyl iodide. By the use of microcrystal tests, and formation of derivatives, it is possible to differentiate biperiden, cycrimine, procyclidine, trihexyphenidyl, and phenyramidol. Photomicrographs are presented as a supplemental and additional parameter for characterization purposes.

VARIOUS techniques for the identification of trihexyphenidyl, cycrimine, and procyclidine hydrochloride salts have appeared in the literature. These include microcrystal tests and color spot tests (1), chromatographic studies (2–4), published infrared spectra (5), and derivatization (6–9).

A few isolated derivatives have been reported for phenyramidol hydrochloride (10–12) and those can be used as an aid to identification. No method for the qualitative estimation of biperiden hydrochloride could be found in the literature.

All of these preparations are potent systemic drugs and are intended for oral or parenteral administration; hence reliable criteria for their identification and differentiation are of great importance to the forensic chemist and toxicologist. It is the purpose of this investigation to develop a comprehensive series of several physical reference criteria which can be utilized to positively identify these compounds in the least amount of time.

EXPERIMENTAL

Apparatus.—Fisher-Johns melting point apparatus; Beckman IR-5A infrared spectrophotometer; Bausch & Lomb biological microscope; Metrohm potentiograph model E336.

Compounds Investigated.—Phenyramidol HCl or 2-β-hydroxyphenethylamino-pyridine HCl; biperiden HCl or [α-bicyclo (2.2.1)-hept-5-en-2-yl-α-phenyl-1-piperidino-propanol HCl]; cycrimine HCl or 1-cyclopentyl-1-phenyl-3-piperidino-1-propanol HCl; procyclidine HCl or 1-cyclohexyl-1-phenyl-3-pyrrolidino-1-propanol HCl; trihexiphenidyl HCl or 1-cyclohexyl-1-phenyl-3-piperidino-1-propanol HCl.

Reagents and Solutions.—All reagents used were A.C.S. grade or the highest grade commercially available. Aqueous and ethanolic (95%) solution of: trihexyphenidyl HCl, 0.25, 0.5, 1%; cycrimine HCl, 0.25, 0.5, 1%; procyclidine HCl, 0.25, 0.5, 1%; phenyramidol HCl, 0.25, 0.5, 1%; biperiden HCl, 0.05, 0.1%; aqueous Reinecke salt solutions, 0.1, 0.25, 0.5, 1.0%; aqueous and ethanolic (95%) picric acid solutions, 0.25, 0.5, 1.0%, and in acetone, 0.5, 1%; aqueous and ethanolic (95%) styphnic acid solutions, 0.16, 0.32, 0.64, and 0.25, 0.5, 1.0%, respectively; aqueous chloroplatinic acid solutions, 0.25, 0.5, 1.0%; ethanolic (95%) solutions of picrolonic acid, 0.25, 0.5, and 1.0%. These solutions were stored in 15-ml. amber dropper bottles.

Formation of Derivatives

All purified derivatives were dried in a vacuum desiccator over phosphorus pentoxide at room temperature for 24 hr. before the final melting point was taken on a Fisher-Johns melting point apparatus. Melting points were corrected by a calibration graph prepared from U.S.P. melting point reference standards.

Picrates.—The general procedure for preparation of picrates was that employed by Shriner *et al.* (13). The crude material was recrystallized from 95% ethanol and the equivalent weights determined by the titration technique of Clark and Wang (14). The data obtained, together with the elemental analyses (C, H, and N) verified the purity and identity of the derivatives.

Reineckates.—The procedure of Chatten and Levi (15) was used. The resulting derivatives were recrystallized by adding approximately 5–10 ml. of methanol and sufficient acetone dropwise to solubilize the derivative if it had not already dissolved. Water was then added dropwise with agitation until

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a turbidity was seen to persist. All recrystallizations were performed without the aid of heat.

Purity and identity of the reineckates was established by elemental analyses (C, H, N, and Cr).

Tetraphenylborates.—The general technique of Koehler and Feldmann (16) was used. Products were recrystallized from methanol or methanol/acetone by adding water dropwise with agitation until a turbidity persisted and then cooled.

Equivalent weights of the tetraphenylborates were determined by the nonaqueous titration procedure of Chatten *et al.* (17). These data, together with the elemental analyses (C, H, N), confirmed the identity and purity of these derivatives.

Methiodides.—The procedure by Cheronis and Entrikin (18) for the preparation of methiodide derivatives was used. Products were recrystallized from isopropanol or acetone–ether.

Purity and identity was confirmed by elemental analyses (C, H, N).

Chloroplatinates.—The method outlined by Wild (19) for the preparation of chloroplatinic acid derivatives was modified and adapted for these amine hydrochloride salts.

Biperiden hydrochloride, being almost insoluble in water, was dissolved in a minimal amount of methanol to which a slight excess of aqueous chloroplatinic acid was added with stirring. The mixtures were allowed to stand for 20 min. in an ice bath and then filtered. The resulting products were purified by washing well with cold water.

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

Infrared Spectra

Infrared spectra of the parent compounds and derivatives were measured by the potassium bromide pellet technique.

Preparation of Photomicrographs

One drop of an aqueous or alcoholic solution of the amine muscle relaxant salt was placed on a microscope slide and 1 drop of a reagent solution was added. These were mixed well and covered with a cover glass. For the potassium iodide test, 1 drop of an aqueous solution of the parent compound was placed on a microscope slide, and a few particles of finely ground potassium iodide were sprinkled over the surface of the drop.

In all instances, the time of crystal formation was noted and the photomicrographs were taken before the slide became dry.

RESULTS AND DISCUSSION

Formation of Derivatives

The melting ranges of the derivatives prepared from the compounds under investigation, together with previously reported literature values, are presented in Table I.

Picrates.—A review of the literature has revealed that relatively few picrates have been reported for the basic amino muscle relaxants. Delaby *et al.* (11) have reported the preparation of the picrate of phenyramidol with a melting point of 151.0–152.0°.

Trihexyphenidyl and biperiden picrate were not obtained in a crystalline state. These products initially formed an oily mass, and repeated crystallization attempts from various solvents (ethanol, methanol, acetone) proved unsuccessful.

The other picrate derivatives were obtained in good yield, were easily purified, and had sharp, well distributed, characteristic melting points.

Reineckates.—In every instance, the amine muscle relaxants studied formed the anhydrous reineckate. This was verified by elemental analyses (carbon, hydrogen, nitrogen) and the gravimetric determination of chromium calculated as Cr_2O_3.

All reineckates prepared in this program were observed to have a reasonably sharp decomposition range. However, some overlap in the melting ranges of the derivatives was evident, thus necessitating the preparation of other derivatives for conclusive identification.

Tetraphenylborates.—It has been stated that washing TPB salts free of excess reagent, and drying under vacuum yields compounds which are sufficiently pure for characterization purposes (20, 21). In this investigation, the TPB derivatives were recrystallized to obtain well-defined crystalline products for infrared spectra. Crane (21) has shown several TPB salts to be heat labile, and for this reason the derivatives were prepared and recrystallized at room temperature.

Since good yields of easily purifiable product were obtained and well distributed melting points were noted, the tetraphenylborate salts proved to be highly desirable derivatives for qualitative identification. When titrated in nonaqueous media, excellent quantitative recoveries were obtained for all derivatives.

Methiodides.—The observed melting point of cycrimine methiodide is radically different from that reported in the literature. No apparent reason for this anomaly could be found other than the failure

TABLE I.—MELTING POINTS OF DERIVATIVES OF THE AMINE COMPOUNDS, °C.

Drug	Picrate	Reineckate	Tetraphenylborate	Methiodide	Chloroplatinate
Biperiden		160.0–164.0(dec.)	208.0–209.5	207.0–208.0	142.0–147.0(dec.)
Cycrimine	145.0–146.5	154.0–158.0(dec.)	157.5–159.0	145.0–146.0 235.0–237.0(9)	179.0–182.0(dec.)
Procyclidine	63.5–65.0	149.0–154.0(dec.)	148.0–150.0	204.0–205.0(dec.) 204.0–205.0(6)	199.0–203.0(dec.)
Trihexyphenidyl		159.0–162.0(dec.)	157.0–159.0	208.0–208.5 203.0–204.0(6,8)	132.0–137.0(dec.)
Phenyramidol	153.5–154.5 151.0–152.0(11)	156.0–158.0(dec.)	124.0–126.0	165.5–166.5 164.0–166.0(10)	156–160.0(dec.)

of the workers (9) to confirm identity of the derivative by elemental analysis.

The methiodides were readily prepared and easily purified derivatives with sharp melting points. Their only apparent disadvantage is the overlap in the melting ranges which precludes their use for qualitative identification without the preparation of additional derivatives.

Chloroplatinates.—All of the amino muscle relaxants studied in this investigation were monobasic and formed the 2:1 derivative with the divalent platinic anion.

The literature reveals no record that chloroplatinic acid has been used previously to characterize any of the amino muscle relaxants investigated in this program. All chloroplatinate derivatives decomposed on heating but the decomposition ranges were reproducible and characteristic. Recrystallization was a factor encountered during the preparation of these derivatives. Generally the chloroplatinates were only sparingly soluble in ethanol (95%) or methanol, and any amount of heating resulted in a partial or complete decomposition of the product. In order to avoid this the chloroplatinates were purified by washing well with cold distilled water prior to submission for elemental analyses. Excellent results were obtained and decomposition ranges were characteristic in each instance with no overlap.

Infrared Spectroscopy

Examination of the spectra of the parent compounds provided an additional parameter for differentiation, although the same functional groups (tertiary amino and alcoholic) are present in all of the drugs.

Medium to weak adsorption in the 3500 cm.$^{-1}$ region (OH stretching vibration) and medium absorption throughout the 3050–2850 cm.$^{-1}$ aromatic and aliphatic CH stretching region is common to the picrates in this investigation. Multiple or broad bands in the 2700–2250 cm.$^{-1}$ region due to NH^+ stretching vibrations, overtones, and combinations are also present. Intense bands at 1560 and 1360 cm.$^{-1}$ represent the nitro asymmetrical and symmetrical stretching vibrations, respectively. In the "fingerprint" region two sharp characteristic bands, common to the three spectra, appear at 785 and 740 cm.$^{-1}$.

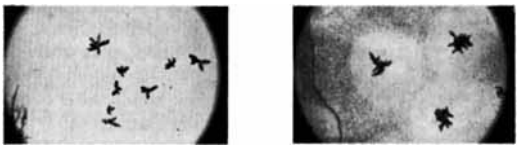
Spectral interpretations appearing in a previous paper of this series (22) are applicable to the reineckates and tetraphenylborates, respectively. However, the methiodide spectra are sufficiently different to merit special mention. These latter spectra exhibit numerous common bands, in keeping with their structural similarity. Strong absorption bands at 3300 cm.$^{-1}$ due to OH stretching vibrations and medium to strong aromatic and aliphatic CH stretching throughout the 3050–2900 cm.$^{-1}$ regions are obvious for all methiodides except that of phenyramidol. In addition, the 8–16 μ region, with its very characteristic CH out-of-plane bending bands in the lower frequencies and especially the 9–14 μ region, is most useful for differentiation of these compounds.

The chloroplatinate spectra were poorly resolved, perhaps due to the damping effect of the anion, and hence were of no value for differentiation of the compounds.

Photomicrography

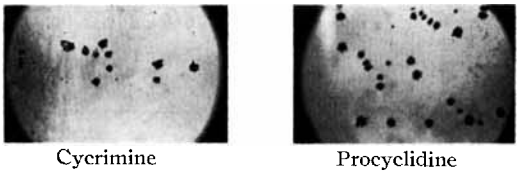
A large number of reagents were employed in an attempt to obtain complete and comparative sets of photomicrographs. However, success was limited to a few of these. All photomicrographs were taken at 50× magnification, and only those depicting distinctive crystal formations are reproduced in Figs. 1–5. It must be understood that the crystalline habits of these compounds are not to be used as the sole criterion for identification, but rather as an adjunct to the other physico-chemical methods previously discussed.

Phenyramidol hydrochloride was the only compound to yield well-defined microcrystals with either picric or styphnic acid and these are presented in Fig. 1. Combinations of these reagents with the other parent compounds yielded oils or oily films which would not crystallize.



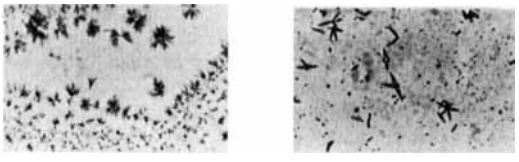
Phenyramidol Picrate — Phenyramidol Styphnate

Fig. 1.—Photomicrographs of the picrates and styphnates.

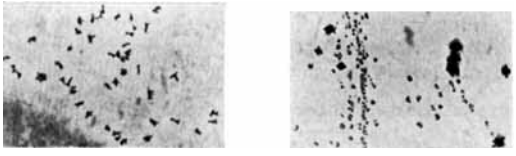


Cycrimine — Procyclidine

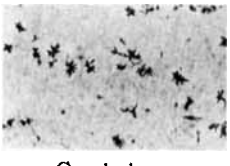
Fig. 2.—Photomicrographs of the chloroplatinates.



Procyclidine — Phenyramidol



Biperiden — Trihexyphenidyl



Cycrimine

Fig. 3.—Photomicrographs of the reineckates.

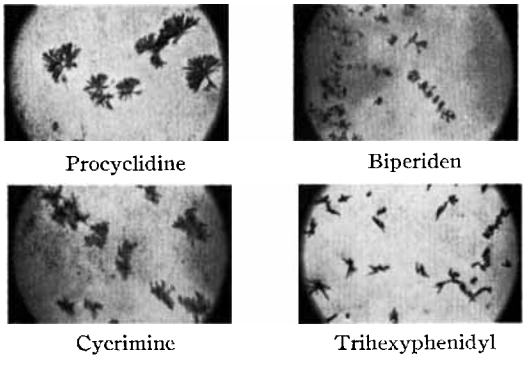


Fig. 4.—Photomicrographs of the picrolonates.

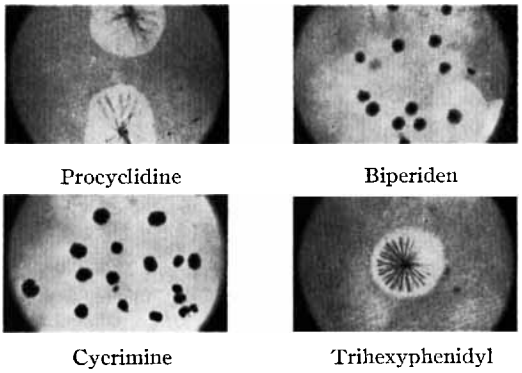


Fig. 5.—Photomicrographs of the hydroiodides.

lonate of phenyramidol could not be obtained in crystalline form under any of the varied conditions employed. The material invariably separated as a golden yellow oil. Clarke (1) has described the morphology of procyclidine picrolonate, and favorable agreement was noted with this investigation.

The method of Hucknell and Turfitt (23) was used for the preparation of the hydroiodides. As illustrated in Fig. 5, satisfactory photomicrographs were obtained for four of the five compounds studied. Phenyramidol yielded a fine amorphous precipitate, but the other compounds gave easily and rapidly prepared microcrystals.

The conditions under which the characteristic microcrystals were formed are summarized in Table II. All solutions are aqueous unless otherwise indicated.

SUMMARY

1. A series of specific physical criteria, by which four amine-containing antiparkinson agents and one amine-containing skeletal muscle relaxant can be positively identified and differentiated, has been presented.

2. Twenty-three derivatives of these drugs have been prepared in a systematic manner, of which 18 have not been reported to date in the literature.

3. The infrared spectra of these derivatives and their parent compounds have been obtained as a further parameter for their qualitative differentiation.

4. A series of photomicrographs have been included in Figs. 1–5, together with a summary of the conditions under which they were formed (Table II).

TABLE II.—AMINE MUSCLE RELAXANTS CHARACTERIZED BY MICROCRYSTALLOGRAPHY[a]

Parent Compd.	Picric Acid	Styphnic Acid	Chloroplatinic Acid	Ammonium Reineckate	Picrolonic Acid	Potassium Iodide
Biperiden HCl	...	...	...	0.1–0.1 (5)	0.1–0.25[b]	0.1 (15)
Cycrimine HCl	...	...	1.0–1.0 (15)	0.5–0.5 (5)	0.5–1.0[b]	0.5 (1)
Phenyramidol HCl	0.5–0.5 (10)	0.5–0.64 (15)	...	0.5–0.5 (20)	...	...
Procyclidine HCl	...	...	0.5–1.0 (3)	0.5–0.5 (5)	0.5–1.0[b] (5)	1.0 (5)
Trihexyphenidyl HCl	...	...	...	0.5–0.5 (10)	0.5–1.0[b] (5)	1.0 (5)

[a] Muscle relaxant concentration (%), reagent concentration (%); numbers in parentheses denote time in minutes when crystals formed. [b] Ethanolic (95%) solution.

Chloroplatinic acid did not prove to be a satisfactory reagent for the identification of all the amine muscle relaxants in this study. Figure 2 shows distinctive, well-defined crystals for only cycrimine and procyclidine. The other compounds formed light amorphous precipitates which were not characteristic. Clarke (1) prepared the chloroplatinate of cycrimine and his description complimented our findings.

Ammonium reineckate proved to be an excellent reagent since characteristic and distinctive crystals were obtained for all five of the amine muscle relaxants. The results are depicted in Fig. 3.

Figure 4 shows that picrolonic acid proved to be a highly satisfactory reagent for characterization of four of the five compounds studied. The picro-

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Some Wax Formulations of Sulfaethylthiadiazole Produced by Aqueous Dispersion for Prolonged-Release Medication

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Drug–wax particles were prepared by pouring heated aqueous solutions of surfactants into melted wax which contained dispersed sulfaethylthiadiazole (SETD). The systems then were slowly cooled to room temperature with stirring. The SETD–wax particles were recovered, washed, dried, and sieved into predetermined mesh size ranges. There appeared to be a direct relationship between drug release rate and average theoretical surface area of the SETD–Glycowax S-932 particles *in vitro*. This relationship was not seen in the SETD–beeswax particles. The dissolution rates of all the mesh ranges of particles studied seemed to be pseudo first order after the first 15 min. of testing. SETD–Glycowax S-932, 50- to 60-mesh particles, showed reasonably good prolonged-release properties in an *in vivo* urinary excretion study.

A NUMBER of methods and techniques have been used in the manufacture of oral dosage forms intended to impart prolonged, sustained, or long-acting therapeutic effect. The production of prolonged release of a drug in a wax matrix by means of aqueous dispersion or an emulsification process is mentioned in the literature, but detailed information is not given. Spray-congealing and spray-drying methods using wax with drugs have received quite a bit of attention.

Yamamoto and Baba (1) describe in their patent an aqueous dispersion method for producing wax pearls containing drug for prolonged-release medication. As an example, a melted wax containing dispersed drug is poured into a 2% polyvinyl alcohol aqueous solution, previously heated to 80°, and stirred until cool. The wax pearls that form are strained, washed with water, and dried. In this patent, several suitable dispersing agents are recommended and a number of wax or wax-like dissolution retardants are illustrated.

Kowarski *et al.* (2) describe a similar process in the preparation of prolonged-release sulfamethazine in small size batches. In this method, 2 parts of Japanese synthetic wax was melted with 1 part of sulfamethazine and poured into a running Waring blender containing cold water. After a few minutes of blending, the resulting suspension was filtered and dried. The particles were then washed with hydrochloric acid to remove sulfamethazine embedded on the outside of the granules, which was determined to be about 58% of the total drug in and on the granule.

The purpose of this investigation was to study some wax formulations of sulfaethylthiadiazole (SETD) produced by an aqueous-dispersion method for prolonged action. Bleached beeswax and Glycowax S-932 were used as the dissolution retarding materials. Beeswax was selected because it is a natural product and has plastic properties, whereas Glycowax S-932, a synthetic wax-like product is brittle. Both materials are edible, and the melting points of both are about the same, approximately 63°.

It was hoped that this study might reveal some

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