Identification and Differentiation of Organic Medicinal Agents II

Muscle Relaxants

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The reactions of some skeletal muscle relaxants (containing an alcohol or carbamate or both functional groups) with phenyl and 1-naphthyl isocyanate, 3,5-dinitrobenzoyl chloride, 3-nitrophthalic anhydride, 2,4-dinitrobenzenesulfenyl chloride, xanthydrol, benzhydrol, and acetic anhydride have been studied. By the use of the melting point data of these derivatives, supplemented with their infrared spectra, it was possible to differentiate mephenesin, methocarbamol, DEP, styramate, phenyramidol, meprobamate, and carisoprodol.

DESPITE the clinical importance of this group of medicinal agents, few techniques are available for the identification of muscle relaxants.

Ultraviolet spectra, color reactions, and photomicrographs have been reported for some 50 tranquilizers, including mephenesin (1), and other workers (2, 3) have used spectrophotometric methods for the identification of a variety of dangerous drugs.

Chromatographic procedures (4–14) comprise most of the literature dealing with the qualitative determination of meprobamate. However, nonspecific color reactions (15, 16), photomicrography (17), and derivatization (18–24) also have been utilized. Closely related carisoprodol has been identified by paper chromatographic and infrared studies (5, 25).

A few isolated derivatives have been reported for DEP (2,2-diethyl-1,3-propanediol)[1] (26–28) and phenyramidol hydrochloride (29–31), while no qualitative methods could be found in the literature for methocarbamol or styramate.

EXPERIMENTAL

Apparatus.—Fisher-Johns melting point apparatus; Beckman IR-5A infrared spectrophotometer.

Muscle Relaxants.—Mephenesin, meprobamate, carisoprodol, phenyramidol hydrochloride, styramate, DEP, and methocarbamol.

Reagents and Solutions.—Xanthydrol (practical grade), 3-nitrophthalic anhydride (reagent grade), 3,5-dinitrobenzoyl chloride (reagent grade), benzhydrol (reagent grade), acetic anhydride (A.C.S.), Catalyst T-9 (stannous 2-ethylhexonate from Metals and Thermit Corp., Hamilton, Ontario), phenyl isocyanate (reagent grade), 1-naphthyl isocyanate (reagent grade), and 2,4-dinitrobenzenesulfenyl chloride (reagent grade).

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[1] Marketed as Prenderol.

Formation of Derivatives

All of the purified derivatives were dried in a vacuum desiccator over phosphorus pentoxide at room temperature for 24 hr. before the final melting points were taken on a Fisher-Johns melting point apparatus. (See Table I.) Identity and purity of all derivatives was confirmed by elemental analyses (C, H, and N).

Phenyl Carbamates and 1-Naphthyl Carbamates.—The Reed *et al.* (32) quantitative phenyl isocyanate method for determination of hydroxy equivalent weights was modified slightly and used as follows. About 250 mg. of the muscle relaxant was dissolved in a minimal amount of toluene. A slight excess of the calculated amount of isocyanate was added by pipet along with 1 drop of Catalyst T-9. The solution was warmed slightly and set aside for about 1 or 2 hr. until crystals of the carbamate formed. Occasionally it was necessary to concentrate the solution on a water bath to help induce crystallization. Styramate was found to be only sparingly soluble in toluene. This compound was suspended in about 25 ml. of toluene, and the calculated amount of isocyanate and catalyst was added. The suspension was periodically heated and stirred during the next hour. It then was allowed to remain at room temperature for about 2 hr. to complete the reaction. Derivatives were recrystallized from 95% ethanol.

3,5-Dinitrobenzoates.—The general method of Cheronis and Entrikin (33) and Katz and Keeney (34) were combined and modified to give the following procedure. A minimal amount of toluene was used to dissolve about 250 mg. of the muscle relaxant. A slight excess of the calculated amount of 3,5-dinitrobenzoyl chloride was dissolved in a minimal amount of toluene in a separate flask. The two solutions were poured together slowly with stirring, and a slight excess of pyridine was added. The solution was mixed, tightly stoppered, and maintained at 40° for 30 min. At the end of

Table I.—Derivatives of Muscle Relaxants[a]

Drug	Stoichiometry Drug–Reagent	Phenyl Carbamates	1-Naphthyl Carbamates	3,5-Dinitrobenzoates	Acid 3-Nitrophthalates	2,4-Dinitrobenzenesulfenates	Xanthyl	Acetyl	Diphenylmethyl
Mephenesin	1:1	...	108.0–109.0	121.5–122.5	...	...	...	...	...
	1:2	121.0–122.5	182.5–184.0	...	...	158.5–159.5	...	...	...
DEP	1:1	...	...	...	205.5–206.5	...	...	...	...
	1:2	136.0–137.0	211.5–213.0	142.5–143.5	...	...	...	...	...
Methocarbamol	1:1	134.5–135.0	87.0– 88.5	146.5–147.5	175.5–177.5	199.5–201.0	137.5–138.0	84.0– 85.0	...
	1:2	...	...	...	...	...	...	...	...
Styramate	1:1	124.5–126.0	133.0–134.0	...	187.0–189.5	181.0–182.5	165.5–167.0	96.5– 98.0	...
	1:2	...	...	...	...	...	...	...	...
Meprobamate	1:1	...	...	...	...	...	188.0–189.0	127.5–128.5	108.0–109.0
	1:2	...	...	...	...	...	182.0 (22)	125.0–130.0 (19, 20)	
							188.0–189.0 (24)		
Carisoprodol	1:1	...	...	...	...	...	141.0–142.5	87.0– 88.5	95.5– 97.0
Phenyramidal	1:1	91.5– 93.0	152.5–153.5	...	194.5–195.5	111.5–113.0	...	...	...

[a] Temperatures in °C.

the reaction time, the toluene and the pyridine were removed under reduced pressure on a water bath. The residue was washed briefly with 5 ml. of 2.0% sodium carbonate and then with three 5-ml. portions of water. The residue was recrystallized from a minimal amount of hot methanol or ethanol.

Acid 3-Nitrophthalates.—The general procedure for the preparation of acid 3-nitrophthalates as outlined by Shriner *et al.* (35) was used, and the resulting derivatives were recrystallized from 95% ethanol.

2,4-Dinitrobenzenesulfenates.—The procedure used by Kharasch *et al.* (36) was followed and the resulting derivatives were recrystallized from a minimal amount of hot methanol or equal volumes of methanol and benzene.

For the sulfenyl derivative of phenyramidol, the procedure as outlined by Wild (37) for the formation of amides of sulfenic acid was used.

Xanthyl Derivatives.—The general procedure of Dechene (24) was employed. If crystallization did not occur in a 10-hr. period, the solution was concentrated under vacuum, and then cooled to induce crystallization. The xanthyl derivatives were washed well with distilled water and recrystallized from hot methanol.

Diphenylmethyl Derivatives.—The general procedure for the characterization of amides by the formation of diphenylmethyl derivatives, as outlined by Cheeseman and Poller (38), was used for the preparation of these derivatives. The products were recrystallized from hot ethanol (95%).

Acetyl Derivatives.—The general procedure employed in the B.P. (20) and U.S.P. (19) for the preparation of the diacetyl derivative of meprobamate was utilized in this investigation.

The resulting derivatives were filtered, washed well with water, and recrystallized from ethanol–water.

Infrared Spectra.—Infrared spectra of the compounds were measured by the potassium bromide pellet technique.

DISCUSSION

Derivatization

The derivatives prepared from the muscle relaxants fall into two categories: (*a*) alcoholic group and (*b*) carbamate group.

Table I presents a summary of the derivatives prepared, together with their melting ranges and previously reported literature values. Only in those instances where the muscle relaxant possessed both functional groups was it possible to prepare a large number of derivatives.

Phenyl and 1-Naphthyl Carbamates.—Most alcohols react readily with isocyanates, according to the following general equation, to give an almost theoretical yield of the corresponding urethan or carbamate (37):

$$R—OH + R'—N=C=O \ldots . R'—NH—COOR$$

The alcohol should be virtually anhydrous, as water reacts with the isocyanate reagent forming the corresponding symmetrical diaryl urea. Isocyanates usually are restricted to the characterization of primary and secondary alcohols, as the majority of tertiary alcohols are dehydrated to olefins, or do not react, under the conditions used.

Since many of the muscle relaxants are 1,2- or 1,3-propanediol derivatives most of them contain at least one free primary or secondary hydroxyl group. A review of the literature has revealed that the carbamate ester has been prepared for many of these compounds (39–41), but the phenyl carbamate and 1-naphthyl carbamate esters have not been synthesized.

It was possible to obtain a phenyl or 1-naphthyl carbamate ester for each of the compounds containing a primary or secondary alcoholic group. However, the monophenylcarbamate derivative of mephenesin could not be isolated in accordance with the procedure employed in this paper.

The isocyanate–alcohol reaction was catalyzed by stannous 2-ethylhexanoate (obtained from Metals and Thermit Corp., Hamilton, Ontario, Canada). The catalyst has been used successfully by Reed *et al.* (32) during a study of a phenyl isocyanate method for the determination of the hydroxyl equivalent weight of polyoxyalkylene compounds. Smith (42) has made extensive catalytic investigations for the formation of urethans using this metal salt catalyst, and has found that the catalyst is applicable to most isocyanate–alcohol reactions.

Primary and secondary amines can be characterized by the formation of substituted ureas through a reaction with some aryl isocyanate. Since phenyramidol contains both a secondary hydroxyl and secondary amine functional group, it is possible that the aryl isocyanates reacted with either the hydroxyl or the amine functional group. The elemental analyses for the carbamate derivatives of this compound showed the formation of a 1:1 product.

3,5-Dinitrobenzoates.—Katz and Keeney (34) found that low concentrations of pyridine were required for maximum ester formation, while high pyridine concentrations depressed the yield. Thus only a slight excess of the calculated amount of pyridine required to combine with the HCl produced in the reaction was added.

Many of the derivatives (*i.e.*, mephenesin di-3,5-dinitrobenzoate, styramate 3,5-dinitrobenzoate, and phenyramidol 3,5-dinitrobenzoate) could not be crystallized upon repeated attempts in various solvents (methanol, ethanol, acetone), and an oily mass was obtained in all instances.

Acid 3-Nitrophthalates.—The acid 3-nitrophthalate esters were prepared for all the muscle relaxants containing a primary or a secondary hydroxyl group, with the exception of mephenesin. Mephenesin (mono and di) acid 3-nitrophthalate esters could not be isolated as crystalline products. In each instance, repeated crystallization attempts from various solvents (ethanol, methanol, acetone) resulted in a gummy oily mass.

Since 3-nitrophthalic anhydride can react with primary or secondary amines as well as with the alcoholic functional group, the phenyramidol acid 3-nitrophthalate ester proved to be of some interest. Elemental analysis of the derivative showed that the product was the 1:1 compound. To ascertain whether the reagent had attacked the hydroxyl or the amino group, phenyramidol base and the derivative were titrated potentiometrically in nonaqueous media according to the procedure of Clair and Chatten (43). In each instance the potentiometric titration curves revealed only one end point, and thus both compounds were titrating on a 1:1 basis. Hence, it is postulated that the 2-amino group is being titrated in both instances. If the 2-amino group had been substituted, the relative basicity of the derivative would have changed from that of the parent phenyramidol base, and no potentiometric end point would have resulted.

2,4-Dinitrobenzenesulfenate Derivatives.—Pyridine greatly facilitates formation of the 2,4-dinitrobenzenesulfenate esters, thereby permitting a general technique for the characterization of the alcoholic group (36).

The benzenesulfenate esters could be prepared for the majority of the muscle relaxants containing a primary or secondary hydroxyl group. Mephenesin mono-2,4-dinitrobenzenesulfenate and DEP di-2,4-dinitrobenzenesulfenate esters were the only anomalous derivatives that could not be isolated in a crystalline state.

Since sulfenyl halides can react with primary and secondary amines as well as with the alcoholic function, the phenyramidol derivative was treated in exactly the same manner as the previously mentioned phenyramidol acid 3-nitrophthalate. The derivative was titrated potentiometrically in nonaqueous media and found to titrate 1:1, thus indicating that the sulfenyl halide had not attacked the 2-amino but rather the hydroxyl group.

Xanthyl Derivatives.—Roth and others (22) have characterized meprobamate by means of its dixanthyl derivative and reported a melting point of 182°, while Dechene (24) has reported the same derivative with a higher melting range of 188 to 189°.

These derivatives of the carbamates were obtained in good yields, easily purified, and had well-distributed melting points.

Diphenylmethyl Derivatives.—As an additional parameter, it was decided to investigate the applicability of benzhydrol as a reagent for the preparation of derivatives of the primary carbamate moiety.

Diphenylmethyl derivatives of styramate and methocarbamol could not be isolated in crystalline form, although repeated attempts were made from various solvents (methanol, ethanol, benzene, and acetone).

Good yields of a characteristic compound were obtained for two of the carbamate muscle relaxants, although considerable difficulty was encountered with carisoprodol. Melting points were sufficiently sharp to permit differentiation of the two parent compounds.

Acetyl Derivatives.—Acetic anhydride has been used by the U.S.P. (19) and B.P. (20) to prepare the diacetyl derivative of the carbamate moieties found in meprobamate. In an attempt to expand this approach, it was decided to investigate the preparation of acetyl derivatives of the other muscle relaxants which contain this same functional group.

Styramate and methocarbamol both contain a secondary hydroxyl group as well as a primary unsubstituted carbamate group. The elemental analyses of the derivatives indicated that the parent compounds had been attacked by two acetyl groups. In an effort to substantiate this, the integrated NMR spectra were determined for these two derivatives, and the integration curves revealed the presence of 19 protons for the methocarbamol

derivative and 15 protons for the styramate derivative. This information complimented the elemental analyses data, by confirming that the investigation had yielded the 2:1 products. Thus, in the case of methocarbamol and styramate, the acetyl group had attacked both the secondary hydroxyl and the primary carbamate nitrogen.

Infrared Spectra

Examination of the spectra of the parent compounds revealed structural features reflecting the presence of specific functional groups.

The phenyl and naphthyl carbamates exhibited numerous common bands, in keeping with that derivative. Weak to medium absorption bands throughout the 3400–3200-cm.$^{-1}$ region due to NH stretching vibrations and weak absorption throughout the 3050–2850 cm.$^{-1}$ aromatic and aliphatic CH stretching region is generally common to all spectra. A strong amide I band (carbonyl absorption of the carbamate moiety) was present in the 1735–1690-cm.$^{-1}$ region. The 8–16 μ region was very characteristic and was most useful for differentiating these compounds.

Mephenesin 3,5-dinitrobenzoate showed a weak to medium band at 3500–3400 cm.$^{-1}$ due to the OH stretching of the hydroxyl group present. Methocarbamol 3,5-dinitrobenzoate exhibited two modes at 3500 and 3400 cm.$^{-1}$ due to asymmetric and symmetric NH stretching vibrations of the primary carbamate grouping. Ester carbonyl absorption was indicated by a strong band at 1725 cm.$^{-1}$. Strong bands at 1540 and 1350 cm.$^{-1}$ represented the nitro asymmetrical and symmetrical stretchings, respectively. Strong doublet modes at 730 and 710 cm.$^{-1}$ were characteristic of the 3,5-dinitrobenzoates in this investigation.

The spectra of the 3-nitrophthalates exhibited many complimentary bands as exemplified by the absorption modes at 1835 and 1700 cm.$^{-1}$ due to the carbonyl moieties found in the ester and carboxylic acid groups. Since DEP acid 3-nitrophthalate is the monoderivative, it retained strong absorption at 3450 cm.$^{-1}$ due to the primary hydroxyl group present. Methocarbamol and styramate acid 3-nitrophthalate both exhibited two free NH stretching modes near 3400 and 3250 cm.$^{-1}$, corresponding to the asymmetric and symmetric motions of the hydrogen atom in the carbamate moiety. The OH stretching frequency of the acid groups occurred as broad bands with a series of minor peaks over the 3000–2500 cm.$^{-1}$ regions. The O—H out-of-plane bending of an acid dimer was indicated by a relatively broad band of medium intensity at 920 cm.$^{-1}$.

Methocarbamol and styramate 2,4-dinitrobenzenesulfenate esters both showed characteristic bands originating in primary carbamate NH stretching modes (3420 and 3300 cm.$^{-1}$). In addition, the carbonyl absorption of the carbamate resulted in an amide I band at 1725 cm.$^{-1}$ for these derivatives. All spectra showed an intense band at 1340 cm.$^{-1}$ representing the nitro symmetrical stretching vibrations.

All spectra of the xanthyl derivatives revealed a single absorption at 3300 cm.$^{-1}$ due to the secondary carbamate NH stretching vibration. Xanthyl methocarbamol and xanthyl styramate showed OH stretching at 3500 and 3400 cm.$^{-1}$, respectively. Medium bands at 2950 cm.$^{-1}$ for the carisoprodol and meprobamate derivatives indicated alkane CH stretching frequencies. A strong amide I band (carbonyl absorption) at 1780–1760 cm.$^{-1}$ was obvious in all spectra.

The aryl ether portion of the xanthyl moiety demonstrated a strong band at 1250 cm.$^{-1}$ due to the =C—O stretching vibration for all derivatives. A strong broad band was evident at 750 cm.$^{-1}$ for all xanthyl derivatives studied.

Upon examination of the spectra of the diphenylmethyl derivatives, medium bands in the 3400–3300-cm.$^{-1}$ range were found to be characteristic for these compounds due to NH stretching vibrations. In addition, the 8–16-μ region was most useful for differentiating these compounds.

A weak to medium NH stretching absorption at 3300–3200 cm.$^{-1}$ was common to all spectra of the acetyl derivatives, while the carbonyl absorption of each particular compound was extremely characteristic. The methocarbamol derivative revealed three carbonyl absorptions (1750, 1725, and 1695 cm.$^{-1}$); styramate and carisoprodol derivatives exhibited two carbonyl absorptions at 1750–1725 and 1740–1680 cm.$^{-1}$, respectively; and the meprobamate derivative appeared to have only a single broad carbonyl absorption at 1730 cm.$^{-1}$.

The acetate ester found in both styramate and methocarbamol gave a typical C—O stretch at 1235 and 1250 cm.$^{-1}$, respectively.

The infrared spectra of the parent compounds and the derivatives were found to afford a useful parameter for the differentiation of this heterogeneous group of medicinal agents.

SUMMARY

A series of specific physical data by which seven of the newer muscle relaxants can be positively identified and differentiated has been presented.

Thirty-two derivatives of these drugs have been prepared in a systematic manner, of which 30 have not been reported to date in the literature.

The infrared spectra of these derivatives and their parent compounds have been prepared, and their pertinent spectral characteristics have been indicated or discussed.

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Nonclassical Antimetabolites XXII

Simulation of 5′-Phosphoribosyl Binding V. Inhibition of Succinoadenylate Kinosynthetase by 6-Mercapto-9-purinylalkanoic Acid Derivatives of 4- and 5-Aminosalicylic Acid

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4- and 5-(6-Mercapto-9H-purine-9-ylvaleramido)salicylic acids (VII and XII) were relatively good inhibitors of succinoadenylate kinosynthetase, being about one-fourth as effective as thioinosinic acid (IX). The only further structural change that allowed retention of inhibition was variation of the valeryl bridge. Removal of the phenolic hydroxyl of VII, replacement of carboxyl group of XII by nitro, or replacement of the salicylic acid moiety by γ-butyric acid led to a decrease in inhibitory properties, thus indicating that both the phenolic hydroxyl and the carboxyl of the salicylate moiety are complexed with the enzyme. The binding of VII and related molecules was finally traced to the acylamino salicylate moiety, and there was no purine binding. Whether the salicylate moiety is simulating the enzyme binding of the phosphate moiety cannot as yet be certain, but appears unlikely.

ALTHOUGH the simulation of the binding of the phosphate moiety of a nucleotide such as IX or X by a more weakly ionized moiety is a goal worthy of pursuit (1–4) for its utility in chemotherapy (1), the solution of this problem is not as simple as the initial results portended (1). 9H-Adenine-9-ylvaleric acid could mimic the ability of 5′-adenylic acid to inhibit both lactic dehydrogenase and glutamic dehydrogenase (1), but 1-uracilvaleric acid failed to mimic 2′-deoxyuridylate (X) in its binding to thymidylate synthetase (2); the latter result led to two detailed studies, namely, on the relative contribution of phosphate *versus* other oxygen functions of the 5′-phosphoribosyl moiety of 5′-adenylic acid when it inhibits succinoadenylate kinosynthetase (3) and on whether the phosphate moiety would complex to enzymes through hydrogen bonds only (4). It was concluded (4) that the most likely mode of binding of phosphate to an enzyme was by one anionic–cationic interaction and one hydrogen bond. There are four such possible modes of binding (I–IV), although III is merely an ionized form of II.

It is possible for the salicylate structure to

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