Table V—Hydrochlorides of 5-Diethylaminoacetamido-2-arylimino-3-aryl-4-thiazolidones and Their Local Anesthetic Activity

R	R′	Yield, %	M.p., °C.	Formula	N, % Calcd.	N, % Found	Onset of Anesthesia (min.) with Administration of Anesthetic[a] in Hydrochloric Acid of Strength 0.05 *N*	0.1 *N*	0.2 *N*
o-Chlorophenyl	*m*-Tolyl	70	164	$C_{22}H_{25}ClN_4O_2S.HCl$	11.64	11.54	15.45	21.30	22.00
m-Chlorophenyl	*m*-Tolyl	65	150	$C_{22}H_{25}ClN_4O_2S.HCl$	11.64	11.50	36.30	38.00	38.30
o-Methoxyphenyl	*m*-Tolyl	50	146	$C_{23}H_{28}N_4O_3S.HCl$	11.75	11.66	26.10	26.40	26.40
m-Methoxyphenyl	*m*-Tolyl	60	162	$C_{23}H_{28}N_4O_3S.HCl$	11.75	11.63	27.00	27.20	27.20
o-Tolyl	*m*-Tolyl	61	138	$C_{23}H_{28}N_4O_2S.HCl$	12.16	12.01	34.00	34.55	36.00
m-Tolyl	α-Naphthyl	65	157	$C_{26}H_{28}N_4O_2S.HCl$	11.28	11.17	35.40	38.20	39.30
m-Tolyl	β-Naphthyl	55	201	$C_{26}H_{28}N_4O_2S.HCl$	11.28	11.11	34.50	36.20	39.00
Procaine hydrochloride[b]		—	—	—	—	—	33.00	34.20	34.20

[a] Concentration of anesthetic, 0.1%. [b] Procaine hydrochloride was used as such.

Pharmacological Screening—Adopting the frog sciaticplexus method (11), the local anesthetic activity of these hydrochlorides was tested on frogs and the time of onset of anesthesia, *i.e.*, the time for which a given concentration (0.1%) of the local anesthetic failed to provoke withdrawal of feet is also reported in Table V.

Pharmacological screening of these compounds has shown that the hydrochlorides of 5-diethylaminoacetamido-3-*m*-tolyl-2-*o*-chlorophenylimino-, *o*-methoxyphenylimino-, and *m*-methoxyphenylimino-4-thiazolidone were the most potent local anesthetics among the compounds reported, as they required less time for the onset of anesthesia than the standard substance, procaine hydrochloride, at 0.1% concentration in 0.7% of saline solution.

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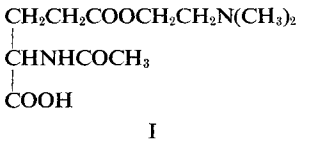
Synthesis of *N*-Acetyl-DL-glutamic Acid 5-Dimethylaminoethyl Ester

G. F. TAMAGNONE and F. De MARCHI

Abstract □ *N*-Acetyl-L-glutamic acid reacted with dimethylaminoethanol in the presence of dicyclohexylcarbodiimide to give *N*-acetyl-DL-glutamic acid 5-dimethylaminoethyl ester (I), which furnished N^2-acetylglutamine by ammonolysis. Compound I could not be obtained by transesterification of *N*-acetyl-L-glutamic acid 5-methyl ester with dimethylaminoethanol, whereas L-pyroglutamic acid salt (III) was obtained from L-glutamic acid 5-methyl ester and dimethylaminoethanol.

Keyphrases □ *N*-Acetyl-DL-glutamic acid 5-dimethylaminoethyl ester—synthesis □ TLC—identity □ IR spectrophotometry—identity, structure

Various dimethylaminoethanol salts have been reported for their CNS-stimulant properties (1–3); particularly, the salt with acetyl-L-glutamic acid which has been recently introduced in Europe as a psychoenergizer (4). Therefore it seemed interesting to synthesize *N*-acetylglutamic acid 5-dimethylaminoethyl ester (I) for pharmacological evaluation.



I

SYNTHESIS

The following attempts were made to synthesize Compound I:

(1) From L-Glutamic Acid 5-Dimethylaminoethyl Ester (II)—The synthesis of II through transesterification of L-glutamic acid 5-methyl ester with dimethylaminoethanol was claimed by Fabre

(5) and Van Gauwenberghe (6). By employing similar experimental conditions, a compound with the expected analytical values and with melting point and specific rotation very close to those reported by the above-mentioned authors was obtained. However, this compound was not II, but the dimethylaminoethanol salt of L-pyroglutamic acid (III), formed by internal cyclization of L-glutamic acid 5-methyl ester with intramolecular displacement of methanol. An analogous cyclization of L-glutamic acid 5-methyl ester to L-pyroglutamic acid by means of ammonia was reported by Beecham (7). Structure III was proved on the basis of IR and TLC data by comparison with an authentic sample; moreover, its acid and basic moieties could be separated by means of ion-exchange resins, while calcium pyroglutamate was obtained by treatment with calcium hydroxide in ethanolic medium.

(2) From N-Acetyl-L-glutamic Acid 5-Methyl Ester—By transesterification of *N*-acetyl-L-glutamic acid 5-methyl ester with dimethylaminoethanol in the presence of a catalytic amount of sodium, starting products were recovered unchanged for moderate heating and short times of reaction, whereas a prolonged heating at higher temperature caused decomposition.

(3) From N-Acetyl-L-glutamic Acid—By treating *N*-acetyl-L-glutamic acid with dimethylaminoethanol in dimethylformamide solution, using dicyclohexylcarbodiimide as water acceptor, Compound I was finally obtained in 50% yield. The use of dicyclohexylcarbodiimide for synthesizing esters from acids and alcohols was originally reported by Zetzsche and Fredrich (8). Moreover, Buzas and Egnell (9) obtained *N*-acetyl-D,L-glutamic anhydride from *N*-acetyl-L-glutamic acid and dicyclohexylcarbodiimide, and the L-anilide from *N*-acetyl-L-glutamic acid, aniline, and dicyclohexylcarbodiimide; however the position of the amide group was not determined.

Structure and Properties—Structure I was proved on the basis of the following evidence:

(*a*) The presence of a basic moiety was supported by the TLC data and it was quantitatively determined by titration with acetous perchloric acid.

(*b*) The ester function was proved by the positive hydroxamic acid test and by the strong IR absorption at 5.8 μ; moreover, the compound was quantitatively retained by a strong cationic resin, which implied the presence of a basic group linked through a nonsaline bond.

(*c*) A single free carboxylic group was present, as shown by potentiometric titration; it gave a broad IR absorption near 6.25 μ, characteristic of an ionized amino acid (10).

(*d*) The presence of the *N*-acetyl group was substantiated by the IR pattern: NH stretching vibration at 3.1 μ; amide I band at 6.1 μ; and amide II band at 6.4 μ (10).

(*e*) No optical activity was observed; therefore racemization occurred during the synthesis.

(*f*) The 5-ester structure of I was determined by reaction with methanolic ammonia: N^2-acetylglutamine was obtained[1]; if the esterification had taken place at the α-carboxylic group, iso-N^2-acetylglutamine should have been formed.

EXPERIMENTAL[2]

Cyclization of L-Glutamic Acid 5-Methyl Ester to L-Pyroglutamic Acid by Means of Dimethylaminoethanol—A mixture of L-glutamic acid 5-methyl ester (33 g., 0.215 mole), dimethylaminoethanol (70 ml., 0.7 mole) and sodium (0.125 g.) was slowly heated to 95° and so maintained 2 hr. under stirring; the temperature was then slowly brought to 70° and the mixture was kept 3 hr. at this temperature. After cooling, 80 ml. of methanol were added and the solution was treated with charcoal. The solvent and the excess of dimethylaminoethanol were evaporated under reduced pressure at 50–60° and the oily residue was recrystallized from ethanol-ethyl acetate to give 31 g. (70%) of III, white crystals with m.p. 58–61°.

Anal.—Calcd. for $C_9H_{18}N_2O_4$: C, 49.53, H,8.31; N, 12.84. Found: C, 49.10; H, 8.26; N, 12.67.

$\alpha_D^{20°} = -17.2°$ (4.23% aqueous solution). The IR spectrum (KBr) is identical with that of an authentic sample of III. TLC (solvent system: butanol–acetic acid–water 4:1:1, v/v): two spots were obtained; the former ($R_f = 0.54$), corresponding to pyroglutamic acid, detected with sulfuric acid; the latter ($R_f = 0.16$), corresponding to dimethylaminoethanol, detected with Dragendorff reagent. Titration: 200 mg. was dissolved in 10 ml. of water and percolated at 0° on a column containing 2 ml. of ion-exchange resin (Dowex 50 W 8, H^+ form). Percolate and washings were potentiometrically titrated with 0.1 *N* sodium hydroxide: 99.5% pyroglutamic acid was found. Formation of calcium pyroglutamate: 200 mg. of the compound was dissolved in ethanol and stirred 20 min. with 100 mg. of calcium hydroxide; after filtering, ether was added and the calcium salt was obtained in a quantitative yield.

Attempts of Transesterification of *N*-Acetyl-L-glutamic Acid 5-Methyl Ester (II) with Dimethylaminoethanol—The reactions were performed under benzene, toluene, or xylene with an excess of dimethylaminoethanol, in the presence of a catalytic amount of sodium; the solvent was continuously distilled through a Vigreaux column. The reaction mixtures were examined on TLC: a single spot, corresponding to that of the starting material, was observed if benzene or toluene was used; with xylene, a number of decomposition products were obtained.

N-Acetyl-DL-glutamic Acid 5-Dimethylaminoethyl Ester (I)—A solution of *N*-acetyl-L-glutamic acid (18.9 g., 0.1 mole) in dimethylformamide (200 ml.) was cooled to 0°; dimethylaminoethanol (10 ml., 0.1 mole) and dicyclohexylcarbodiimide (20.6 g., 0.1 mole) were added and the reaction mixture was stirred 24 hr. at 0°: a white precipitate formed slowly. After 10 hr. at −10°, the solution was filtered from 21 g. of dicyclohexylurea; the filtrate was concentrated to dryness under reduced pressure at 20–30° and the residue was crystallized from 60 ml. of anhydrous ethanol. By recrystallization from ethanol-acetone (1:1), 13 g. (50%) of I was obtained, colorless needles with m.p. 104–107° dec.; no optical rotation was observed. The IR spectrum (mineral oil) was previously discussed.

Anal.—Calcd. for $C_{11}H_{20}N_2O_5 \cdot C_2H_5OH$[3]: C, 50.96; H, 8.57; N, 9.14. Found: C, 50.52; H, 8.49; N, 9.31.

TLC: I (50 γ) gave single spots with $R_f = 0.14$ and $R_f = 0.27$ in the systems butanol–acetic acid–water (4:1:1, v/v) and butanol–acetic acid–water (2:1:1, v/v), respectively; the spots were detected with Dragendorff reagent or with sulfuric acid. Acid titration (0.1 *N* acetous perchloric acid as titrating solution; acetic acid as solvent; crystal violet as indicator): found, 86.20%; calcd., 84.96%. Alkaline titration (0.1 *N* alcoholic potassium hydroxide as titrating solution; dimethylformamide as solvent; thymol blue as indicator): found, 84.40%; calcd., 84.96%. Titration after treatment with cationic resin: the procedure was the same described for Compound III. The pH value immediately turned alkaline.

N^2-Acetylglutamine from I—A solution of I (1 g.) in 12% methanolic ammonia (30 ml.) was kept 7 days at 40°. The reaction mixture was then evaporated to dryness and the residue dissolved in anhydrous ethanol (25 ml.). The solution was acidified with 4 *N* sulfuric acid (0.9 ml.), boiled, filtered, and evaporated to dryness. The residue was crystallized from anhydrous ethanol to give 0.35 g. (60%) of N^2-acetylglutamine, m.p. 186–188°, without depression of the mixed melting point with an authentic sample; the IR spectra were also identical, and two spots with the same R_f (0.45) were observed on TLC (solvent system: butanol–acetic acid–water 4:1:1; v/v).

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[1] Similarly, *N*-acetyl-L-glutamic acid 5-methyl ester gives N^2-acetyl-L-glutamine by ammonolysis (11, 12).

[2] Melting points were determined in open capillary tubes on a Büchi apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer model 257 spectrophotometer. Thin-layer chromatograms were run on Silica Gel G (Merck A-G Darmstadt).

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Comparison of Two Methods for Measuring Drug-Induced Neurotoxicity

R. DUANE SOFIA

Abstract □ A series of experiments is described in which a rotating and a stationary rod procedure were used to determine the neurotoxic effect of various depressant and antidepressant agents in mice and rats. The results of these studies revealed that the rotating rod technique is more sensitive in detecting drug-induced changes in performance. Observed differences between the two methods were more striking in mice than in rats.

Keyphrases □ Neurotoxicity, drug-induced—measurement □ Rotating, stationary rod—neurotoxicity measurements □ Drug-induced neurotoxicity—measurement method comparison

The neurotoxic effect of psychoactive compounds in both mice and rats has been commonly measured utilizing a rotating rod (rotarod) described by Dunham and Miya (1). Since then several investigators (2–10) have attempted modification of this method to establish the optimum parameters for its standardization. Recently Wright *et al.* (11), introduced a method to measure drug-induced neurotoxicity based on the ability of an animal to traverse a stationary, horizontal rod. Studies reported in this paper were conducted to compare the sensitivity of each procedure in two species of rodents, *i.e.*, mice and rats, as methods for measuring the neurotoxicity of various psychoactive drugs.

EXPERIMENTAL

Rotarod Test—The rotarod apparatus used [a modification of the method described by Dunham and Miya (1)] to test mice (male Swiss-Webster strain, 16–26 g.) consisted of a 2.54-cm. wooden dowel divided into 10 equal spaces of 11.43 cm. each by metal disks of 15.24 cm. in diameter. The rod used to test rats (male hooded Long Evans strain, 180–220 g.) was also a 2.54-cm. wooden dowel but was divided into six equal spaces of 20.32 cm. each by metal disks of 30.48 cm. in diameter. The rotarod speed in both instances was 5 r.p.m. Animals were trained to maintain themselves on the rotating rod for at least 1 min.

Stationary Rod Test—The stationary rod apparatus [a modification of the one described by Wright and others (11)] for mice consisted of a 2.54 cm. diameter metal rod 60.96 cm. in length with a platform at either end of the rod. For rats the metal rod used was also 2.54 cm. in diameter but 101.60 cm. long, again with a platform at either end. Training of animals to walk across the horizontal rod required an initial "nip" or "pinch" of their hindquarters as a stimulus to move after being placed on the platform. Additional pinches were not necessary since the animals learned to move along the rod until training criterion was achieved. In this procedure animals were trained to successfully walk the length of the rod twice within a 1-min. trial.

Procedure—In these studies test drugs were administered intraperitoneally. Mice were tested 15 and 30 min. after injection, while rats were tested 15, 30, and 60 min. postinjection. The additional testing interval given to rats was to insure that the time of peak effect for the drug would not be missed since it is accepted that the rate of metabolism in rats is slower than in mice. A trial, in the rotarod procedure, was considered unsuccessful when an animal fell from the rod more than once in a 1-min. period. In the stationary rod test, failure to traverse the rod in at least two of three trials in the 4-min. period was considered an unsuccessful trial. All animals judged unsuccessful at any one testing interval were said to have displayed neurotoxicity.

Drugs—The drugs studied included: chlorpromazine HCl, chlordiazepoxide HCl, tetrabenazine methanesulfonate, benzquinamide, meprobamate, trifluperidol, sodium pentobarbital, imipramine HCl, and thiazesim HCl. All drugs were either suspended or solubilized in 0.25% methylcellulose and dosed in a volume of 0.1 ml./10 g. mice and 0.2 ml./100 g. rats.

Statistics—The median effective dose (ED_{50}) for neurotoxicity with 95% confidence limits and potency ratios were calculated by the method of Litchfield and Wilcoxon (12) at the time of maximum effect. Ten mice or six rats per dose of test drug and a minimum of three dose levels were used for these calculations.

RESULTS AND DISCUSSION

Table I summarizes the results of this study. The approximate time of peak effect in mice for all the drugs studied was 15 min. while in rats this occurred uniformly at 30 min. Therefore, ED_{50} values in each species were dependent on the time between drug administration and testing. Of the drugs tested in mice, only chlordiazepoxide, meprobamate, and thiazesim do not differ significantly by the two methods. Only for pentobarbital was the stationary rod method in mice significantly more sensitive; however, the observed difference between the methods was small. In rats results obtained with the two methods did not vary significantly for chlorpromazine, chlordiazepoxide, tetrabenazine, benzquinamide, meprobamate, and pentobarbital. In this same species neurotoxic effects of trifluperidol were more sensitive to the stationary rod procedure than to the rotarod test. The observed differences seen frequently in mice and not in rats may be attributable to a species difference. High doses of drugs used in this study when given to mice caused marked depression which resulted in their falling off the rotating rod. On the other hand, when rats were given high doses of the drugs listed in Table I, a rigid catatonic-type depression (excluding chlorproma-