provided the respective components separate sufficiently sharp to permit measurement on the plate.

Determining a satisfactory solvent system can be done qualitatively by finding the R_f values of each component on a silica gel coated glass microscope slide. A 250-ml. beaker covered with aluminum foil makes a satisfactory developing chamber. Development takes about 20 minutes and, in this way, a large number of solvents can be examined rapidly.

In their report, Purdy and Truter (9) estimated all area measurements by using millimeter graph paper. This method is tedious and can result in eye strain. Using viewfoil on which circles and ellipses of known areas are imprinted is much more rapid and convenient. When standard areas were checked by both methods, the resulting differences were 3% or less.

The proposed method is conveniently applicable to pharmaceutical preparations. For example, tablet excipients and diluents do not interfere with the separation of the active constituents or their delineation. In addition, several assays can be performed on multicomponent dosage forms in a normal 8-hour day.

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Novel Decarboxylative *N*-Alkylation Reaction Resulting from Controlled Pyrolysis of Procaine

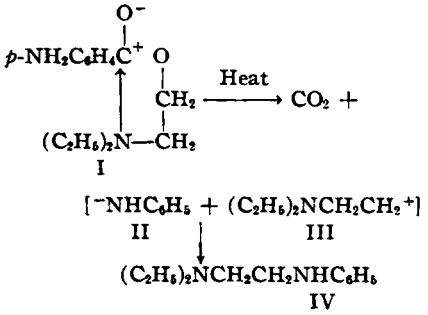
By NATHANIEL GRIER

2-Diethylaminoethyl *p*-aminobenzoate is unstable at elevated temperatures and evolves carbon dioxide, 2-diethylaminoethanol, and *N,N*-diethyl-*N'*-phenylethylenediamine. The pharmacological properties of the diamine correlate with some of the actions of systemic procaine.

AN INCREASED interest has been evidenced in the pharmacological properties of systemic procaine. One active pursuit has been the resolution of conflicting clinical observations reported by various investigators in studies of its long-term administration to humans. Another aspect centers upon the inability of correlating useful clinical effects with the drug's rapid *in vivo* metabolism by hydrolysis to *p*-aminobenzoic acid and 2-diethylaminoethanol. Intravenous procaine has been found effective for the relief of herpetic pain and postherpetic neuralgia (1). Efforts to solve some of the uncertainties include searches for active metabolites and the identification of significant impurities which may be present initially in commercial drug preparations.

With this in mind, a novel decarboxylative *N*-alkylation reaction of procaine which yields carbon dioxide and *N,N*-diethyl-*N'*-phenylethylenediamine is reported. The process was first encountered upon subjecting the ester to moderately elevated temperatures in a study of its manufacture. At temperatures above 200°, preferably in the range 225–235° and under pressures of 20–25 mm., the compound evolves carbon dioxide and easily condensable vapors consisting of a mixture of the diamine and 2-diethylaminoethanol. The aminoalcohol is formed by intermolecular amine-ester self-condensation and is separated readily from the diamine by fractional distillation.

Production of the diamine appears to be initiated intramolecularly by attack of the nucleophilic ternary ester nitrogen atom upon the procaine acyl carbonium ion (I). After loss of carbon dioxide, the aniline anion (II), the result of a proton shift to the ring, and the diethylaminomethylcarbonium ion (III) combine to give *N,N*-diethyl-*N'*-phenylethylenediamine (IV).



Received March 25, 1964, from the Chemical Research Laboratory, Metalsalts Corp., Hawthorne, N. J.
Accepted for publication April 6, 1964.

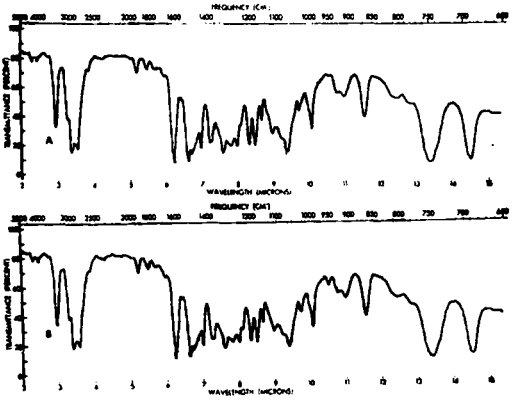


Fig. 1.—Infrared absorption spectra of (top) *N,N*-diethyl-*N'*-phenylethylenediamine, synthetic; (bottom) procaine pyrolysis isolate.

A possible ring alkylate, *p*-amino *N,N*-diethylphenethylamine, $p\text{-}NH_2C_6H_4CH_2CH_2N(C_2H_5)_2$, which could form by interaction of the carbonium ion (III) and *p*-aminophenyl anion, was not detected.

An authentic sample of the aniline *N*-alkylate was prepared by reacting aniline with 2-diethylaminoethyl chloride (2). It was identical to the pyrolysis isolate in boiling point, 131–133° (6 mm.); melting point as the monohydrochloride, 135° (3); melting point as the salicylic acid salt of the *N*-benzoyl derivative (4), 112–113°; and by comparison of chromatograms and infrared spectra (Fig. 1).

Ethyl *p*-aminobenzoate (benzocaine) was unaltered when heated at 235° over a 4-hour period. Cruickshank and Sheehan noted the essential role of intramolecular interactions between functional groups to facilitate decarboxylative acylation of γ- or δ- dialkylamino acids with anhydrides (5).

The pyrolysis of benzoic esters at temperatures above 400° yields benzoic acid and olefins corresponding to the alcohol moiety; as a secondary process the acid decomposes losing carbon dioxide. No recombination of the pyrolytic fragments was reported. Esters which contained at least one β-hydrogen atom were thought to pyrolyze *via* a cyclic transition state in which some carbonium ion character is attributed to the α-carbon atom (6). Dialkylaminopropyl and dialkylaminoisopropyl benzoates decompose at temperatures of 440–450°, forming benzoic acid and the olefinic amines, allydimethylamine and 2-dimethylamino-1-propene; no reference was made to carbon dioxide as a secondary product (7).

A consideration of the pharmacological properties of systemic procaine with those of *N,N*-diethyl-*N'*-phenylethylenediamine reveals areas of similar actions. One of the puzzling effects of intravenous procaine is systemic analgesia.[1] Administration of its water-soluble salts leads to liberation of the active ester base, which in turn is destroyed rapidly by the liver and plasma in humans (8). Essentially, the mechanism is enzymatic hydrolysis; procaine in human plasma completely disappears within 2 minutes in the absence of esterase inhibitors (9).

[1] This property of systemic procaine has been questioned in Keats, A. S., D'Alessandro, G. L., and Beecher, H. K., *J. Am. Med. Assoc.*, **147**, 1761(1951).

The kinetics of hydrolysis were studied in detail by Kalow (10). It is apparent that neither the short-lived ester nor its hydrolytic end products can be the agents responsible for the useful effects; the concentration of nonhydrolyzed drug capable of being achieved in extracellular fluid is too low for peripheral nerve block, and the hydrolysis products are without anaesthetic action. The analgesic properties of the diamine indicate it may be a significant metabolite of procaine, a possible enzymatic decarboxylation product formed in varying amount. A series of potent synthetic analgesics has been described in which the basic structure included *N,N*-dialkyl-*N'*-phenylethylenediamines (11).

In another area, some of the clinical properties ascribed to procaine parallel those of compounds classified as monoamine oxidase inhibitors (12). One of the significant effects, *i.e.*, increased psychomotor activity (13), is of particular interest because it is associated in part with tryptamines, compounds formed *in vivo* by the enzymatic decarboxylation of tryptophanes. These amines may be considered cyclic vinylogs of the *N*-alkylated aniline; a most important member of the group is serotonin or 5-hydroxytryptamine.

N,N-Diethyl-*N'*-phenylethylenediamine is an effective inhibitor of monoamine oxidase in mice measured by the accumulation of serotonin in the brain.[2] A single dose of 50 mg./Kg. body weight was injected intraperitoneally for bioassay, and a second dose was given 16 hours later. The animals were sacrificed 1 hour after and the brain serotonin content determined by the usual analytical fluorescent procedure. The serotonin content of treated animals was compared to that of control animals receiving no compound. In two separate determinations using five animals in each test, brain serotonin ratios of 1.79 and 1.69 compared to controls were obtained, a potency equal to iproniazid[3] in this method. Intact procaine, on the other hand, appears to be a direct antagonist of serotonin (14).

The behavioral stimulant capacity of the diamine was tested in rats and squirrel monkeys at oral dosages up to 90 mg./Kg. body weight. In a conflict schedule involving trained rats, who pressed a lever for food and simultaneously received electric shocks, no significant effects were noted. Similarly, using an avoidance schedule wherein squirrel monkeys had to operate a lever to avoid receiving electric shock, the compound was without promise.

EXPERIMENTAL

Melting points in capillary tubes were determined using an oil bath with Anschütz thermometers. Elemental analyses were performed by Schwarzkopf Laboratories, New York, N. Y. Infrared spectra were obtained with a 0.0025 in. thickness of compound *versus* air in a Perkin-Elmer model 21 spectrophotometer.

Procaine Pyrolysis.—Pure procaine base was prepared by precipitation from an aqueous solution of its hydrochloride using dilute ammonium hydroxide solution. After drying at 40° for 16 hours, it was

[2] Animal bioassays and tests were obtained through the cooperation of Dr. J. M. Sprague and his associates, Merck Sharp and Dohme Research Laboratories, Division of Merck and Co., Inc., West Point, Pa.

[3] Marketed as Marsilid by Hoffmann-LaRoche, Inc. Nutley, N. J.

twice recrystallized from petroleum ether, b.p. 30–60°; m.p. 61–62°.

In a 100-ml. Claisen flask set for vacuum distillation 59 Gm. (0.25 mole) procaine base was held at an internal temperature range of 225–235°, while a pressure of 19 mm. was maintained using a controlled air leak at the vacuum pump. Within a 1-hour period under these conditions 17.8 Gm. of practically colorless liquid was collected in a brine-cooled receiver; the residue in the distillation flask was a gummy yellow solid. Evolution of carbon dioxide throughout the distillation was observed by passing the noncondensed vapor through a Drierite U-tube to a soda-lime trap (indicator type, 4–8 mesh, Fisher Scientific Co.).

Fractionation of the distillate through a 10-cm. Vigreux column at 5 mm. pressure yielded two liquids in nearly equal amounts; the first was collected until an upper vapor temperature of 80° and the second at 133–135°. The lower boiling fraction was identified as 2-diethylaminoethanol and converted to its hydrochloride, m.p. 133–135° (15).

The second liquid was submitted to a more detailed study. Treatment of the residue, a resinous polyamide, with boiling aqueous 5% sodium hydroxide led to the recovery of *p*-aminobenzoic acid on adjustment of pH to 4.0–4.5.

Proof of Structure of Pyrolysate II as N,N-Diethyl-N′-phenylethylenediamine.—The second pyrolysis fraction was converted to a monohydrochloride salt by treatment with dry hydrogen chloride in ether or 99% isopropyl alcohol. The salt so obtained was recrystallized from 99% isopropyl alcohol, m.p. 133–135°.

Anal.—Calcd. for $C_{12}H_{21}ClN_2$: C, 63.02; H, 9.18; Cl, 15.53; N, 12.25. Found: C, 63.15; H, 9.02; Cl, 15.25; N, 11.98.

For comparison, the free base was synthesized by the alkylation of aniline with 2-diethylaminoethyl chloride in absolute alcohol containing excess sodium carbonate (2). On reaction with dry hydrogen chloride it formed a monohydrochloride salt which melted at 133–135° (3). A mixed melting point with the hydrochloride salt of pyrolysate II showed no depression.

The second pyrolysis fraction was characterized further by benzoylation and isolation as a crystalline salicylate.

Pyrolysate II was heated with the calculated quantity of benzoyl chloride at 100° for 24 hours (4).

One equivalent of salicylic acid was added to a 30% solution of the liquid benzoyl derivative in warm benzene. Addition of 0.5 vol. of petroleum ether (b.p. 30–60°) and gradual cooling deposited the colorless crystalline salicylate, which was recrystallized from the same solvent system, m.p. 112–113°.

Anal.—Calcd. for $C_{26}H_{30}N_2O_4$: C, 71.85; H, 6.95; N, 6.45. Found: C, 72.11; H, 6.88; N, 6.50.

N,N - Diethyl - *N′* - benzoyl - *N′* - phenylethylenediamine salicylate was prepared similarly from the synthesized base. This salt melted at 112–113°, a mixed melting point of the two salicylates showed no depression.

p-Amino-N,N-diethylphenethylamine.—Catalytic hydrogenation of *p*-aminobenzyl cyanide in the presence of diethylamine using 10% palladium on barium sulphate yielded the product after removal of the catalyst and fractional distillation, b.p. 115–116° (0.5 mm.) (16).

Chromatographic Comparisons.—Chromatograms were obtained using strips of Whatman No. 1 filter paper in an ascending system. Development included the use of a mixed solvent prepared from 2 vol. *n*-butanol and 1 vol. each glacial acetic acid and water; the time required was 16 hours at 20°. For location of compounds the paper was air-dried, sprayed with a 0.2% ninhydrin in acetone solution, then heated at 110° for 10 minutes.

The synthetically prepared *N,N*-diethyl-*N′*-phenylethylenediamine and *p*-amino-*N,N*-diethylphenethylamine were compared to the second fraction of the pyrolysate. The *p*-substituted aniline, a colorless liquid stored under nitrogen, gave an immediate yellow color on contact with the paper. The other two products remained colorless. Identical R_f values were obtained—0.80—for the ethylenediamine and the pyrolysate, with bluish-green spots on reaction with ninhydrin. An R_f of 0.69 was noted for the *p*-substituted aniline and a yellowish-brown color with ninhydrin. Repeated chromatography trials failed to show its presence in the pyrolysate. The closeness in boiling point of the diamine, 154–158° (17 mm.) (17), and the *p*-alkylated aniline, 154–157° (13 mm.) (16), prevented seperation by distillation.

Ethyl p-Aminobenzoate Pyrolysis Attempt.—The ester was twice recrystallized from ethyl alcohol, m.p. 87.5–88.8°. It was heated at the same internal temperature range (225–235°) as procaine, but a pressure of 65 mm. was selected to maintain reflux of the ester in the Claisen flask without condensation of any formed aniline or *N*-ethylaniline. There was no detectable carbon dioxide evolution over a 4-hour period of heating; the pressure was reduced to 0.8 mm., and the ester recovered quantitatively at a boiling point of 141–143°. The distillate rapidly solidified on cooling and showed no change in melting point.

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