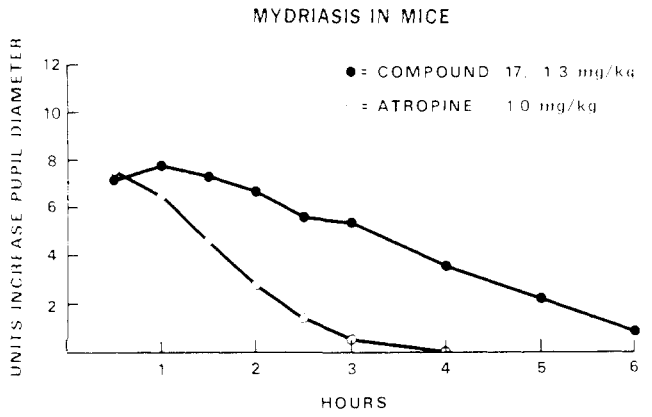

Figure 2.

The 4-halo-substituted compounds **9** and **13** had the largest separation between spasmolytic and lethal effects; **9** was also the most potent and was tested further in comparison to atropine. Compound **9** was not mydriatic in mice and rats, while atropine had a potent effect at doses of 1 mg/kg or less. The antisialogogic activity of **9** was weak in mice, less than $^1/_{100}$th that of atropine. Compound **9** was about one-seventh as potent as atropine in the fecal pellet test. In the charcoal meal test (see Table V), the effects of the two agents were quite different. Atropine produced a maximum of about 50% inhibition at 200 and 400 mg/kg, while **9** produced inhibition ranging from 42 to 94% at doses of 94–185 mg/kg.

Von Oettingen[16] pointed out that the parasympatholytic action of atropine and its analogs was closely connected with the existence of a free alcoholic OH group on the β carbon. The observed virtual abolishment of mydriatic and antisalivary activity among the acrylate type compounds, the main qualitative difference between them and the hydracrylates, would tend to support this idea. The qualitative differences are particularly striking between the acrylates **9**, **13**, and **14** on one hand and the hydracrylates **17–19** on the other.

The acrylates appear to represent a potentially useful class of drugs because they have spasmolytic properties but are nonmydriatic, and thus may not have the side-effect potential of anticholinergic spasmolytics.

TABLE V

INHIBITION OF CHARCOAL MEAL TRANSIT IN RATS

Compd 9		Atropine	
Dose, mg/kg *po*	% inhib of small intestinal transit	Dose, mg/kg *po*	% inhib of small intestinal transit
185	90	400	54
148	64	200	52
118	46	100	38
94	42	25	32
		10	10

Acknowledgments.—We are indebted to Miss Margaret Carroll and her staff for microanalysis, to Mr. Robert North for technical assistance, and to Dr. L. C. Greene for encouragement.

(16) W. F. Von Oettingen, "Therapeutic Agents of Pyrrole and Pyridine Group," Edwards, Ann Arbor, Mich., 1936, pp 141–168.

Arundo donax L. (*Graminae*). Phytochemical and Pharmacological Evaluation

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Five indole-3-alkylamine bases, *viz.*, N,N-dimethyltryptamine, 5-methoxy-N-methyltryptamine, bufotenine, dehydrobufotenine, and bufotenidine, were isolated from the rhizomes of *Arundo donax* L. This is the first reported occurrence of bufotenidine and dehydrobufotenine in a plant species. A defatted ethanolic extract of the rhizomes produced hypotensive and antispasmodic effects against histamine-, serotonin-, and acetylcholine-induced spasms. Bufotenidine showed three main pharmacological actions, *viz.*, antiacetylcholine effect which appears to be more specific against skeletal muscle than against muscarinic sites, histamine release, and uterine stimulant. None of these actions of this compound had been reported previously.

Arundo donax L. (*Graminae*), a tall, stout, perennial shrub, often woody below, is widely distributed in India. A decoction of its rhizomes has been used in the Ayurvedic system of medicine[1] as an emollient and diuretic and is said to stimulate menstrual discharge and to diminish the secretion of milk.

From the leaves of this shrub, Madinaveitia previously reported[2] the isolation of three indolic bases, *viz.*, donaxarine, $C_{13}H_{16}N_2O_2$, mp 217°, gramine, and an amorphous phenolic base. Apart from a positive pine splinter reaction and the fact that it cooccurs with gramine little evidence is available regarding the nature of donaxarine. The incomplete characterization of donaxarine and of the amorphous phenolic base by Madinaveitia, and the reported uses of the rhizomes in the Ayurvedic system of medicine prompted us to reinvestigate the basic constituents of this species.

We have previously reported[3] the isolation of five indole-3-alkylamines, *viz.*, N,N-dimethyltryptamine, 5-methoxy-N-methyltryptamine, bufotenine, gramine,

(1) R. N. Chopra, S. L. Nayar, and I. C. Chopra, "Glossary of Indian Medicinal Plants," C.S.I.R., New Delhi, 1956, p 27.

(2) J. Madinaveitia, *J. Chem. Soc.*, 1927 (1937).

(3) S. K. Dutta and S. Ghosal, *Chem. Ind.* (London), 2046 (1967).

and its N_b-oxide from the leaves of *Arundo donax*. In addition, paper and thin layer chromatography revealed the presence of several quaternary indolic bases. The present paper deals with the chemistry and pharmacology of the indole-3-alkylamines in general and bufotenidine in particular, occurring in the Indian reed.

The utilization of the pH-gradient separation and column chromatography on partially deactivated Brockmann alumina have resulted in the isolation of three previously known[3,4] secondary and tertiary indole-3-alkylamines, *viz.*, N,N-dimethyltryptamine, 5-methoxy-N-methyltryptamine, and bufotenine, and two quaternary indolic bases, *viz.*, bufotenidine and dehydrobufotenine, from the alcoholic extract of the rhizomes of this plant. In addition, the total alkaloidal extract revealed, on paper and thin layer chromatograms, the presence of a number of 5-hydroxyindole bases (color reaction with α-nitroso-β-naphthol reagent[5] positive) of low R_f values. Gramine, the major alkaloid of the leaves,[2,3] was not found in the rhizomes. This is the first reported occurrence of bufotenidine and dehydrobufotenine in a plant species. The only other natural source known for these compounds so far was the toad poison.[6,7]

As the rhizomes contained very little fat the alkaloids were extracted directly with alcohol without prior defatting with petroleum ether. This simple but important modification in the usual extraction procedure kept the formation of a melanin-like polymer to a minimum. The dark brown polymer showed Dragendorff-positive and Ehrlich-negative color reactions. The N analysis of this compound was low, indicating thereby a very high state of oxidation of the polymer. Autoxidation, a serious problem during isolation and preservation of the chloroform-soluble bases also led to similar products which were finally converted to a black melanin.

Pharmacology.—The observations made by us during the pharmacological testing that certain fractions prepared from the rhizomes of *A. donax* produced hypotensive and antispasmodic effects against histamine-, serotonin-, and acetylcholine-induced spasms led us to submit the defatted ethanolic extract of the plant to a more thorough pharmacological screening program. During these investigations, the extract was found to exhibit a strong curare-like action on frog rectus abdominis muscle. It was soon determined that these biological activities resided in the alkaloidal entities contained in the extract.

Detailed pharmacological studies have, however, been made with bufotenidine only as its yield was maximum and as very few studies seem to have been made[8] with this alkaloid. Routine pharmacological methods have been employed to study its action on the cardiovascular system, CNS, smooth and skeletal muscles, and urinary and fecal output.

The drug was found to have characteristic actions on the dog's blood pressure, skeletal muscle, and smooth muscles. It showed no significant effect on barbiturate-induced hypnosis or as an analgetic (tested by rat tail hot wire method) or as an anticonvulsant (against maximal electroshock and pentylenetetrazole-induced convulsions). It showed some cardiotonic activity on the frog's perfused heart but this was not considered of significant importance as it failed to show a similar effect on the hypodynamic heart (produced by perfusing the heart with Ringer's solution containing 25% Ca^{2+}).

Effect on Blood Pressure.—Intravenous administration of the drug (0.2–0.5 mg/kg) produced varying degrees of fall in blood pressure. The fall was sharp and the recovery was slow and gradual. The duration of hypotension after the first dose varied from 0.5 to 1.5 hr. Repeated injections of the same dose of the drug showed tachyphylaxis (Figure 1). Further, the

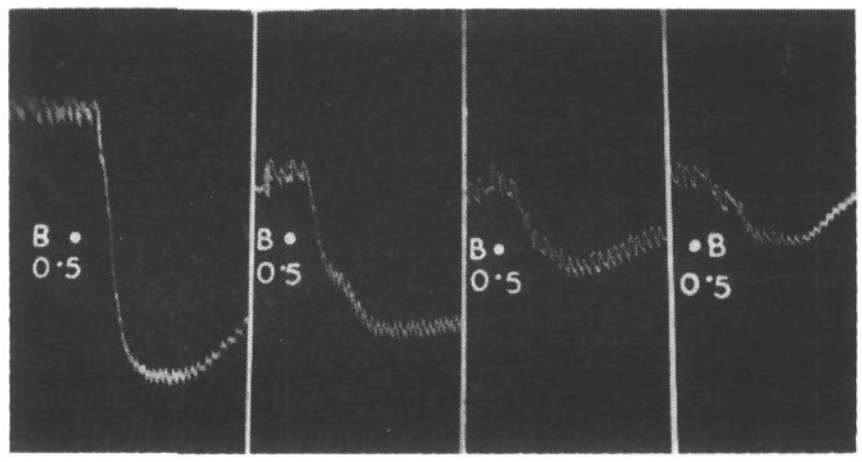

Figure 1.—Effects of repeated doses (0.5 mg/kg) of bufotenidine on pentobarbital-anesthetized dog carotid blood pressure. At the white lines the drum was stopped for 30 min.

hypotensive response of the alkaloid was either absent (Figure 2) or very much reduced if administered after

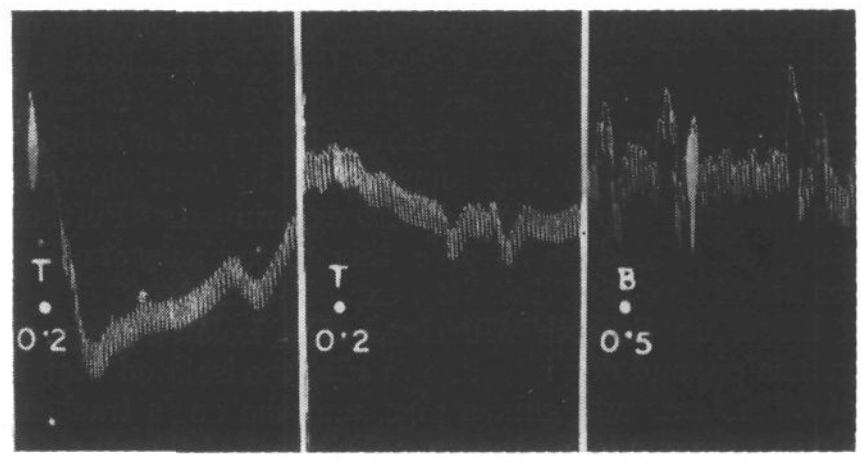

Figure 2.—Effect of bufotenidine (0.5 mg/kg) on pentobarbital-anesthetized dog blood pressure after prior administration of two doses (0.2 mg/kg) of *d*-tubocurarine. At the white lines the drum was stopped for 30 min.

prior treatment of the animal with one or two doses of *d*-tubocurarine (0.2–0.4 mg/kg). The reverse phenomenon was also observed, *i.e.*, prior administration of the alkaloid reduced or abolished the hypotensive response of *d*-tubocurarine. Interestingly, after tachyphylaxis the hypotensive response could be produced if the alkaloid was administered after slow histamine perfusion (5 μg/ml/min for 30 min) which itself did not produce any appreciable hypotensive response (Figure 3). These observations suggest that the mode of hypotensive action and tachyphylaxis are due to histamine release from some stores which could be replenished by the slow histamine perfusion to be again released by a histamine releaser.

Effect on Skeletal Muscle.—The alkaloid was found to block specifically the acetylcholine-induced (5 μg/ml)

(4) S. Ghosal and B. Mukherjee, *Chem. Ind.* (London) 1800 (1964); 793 (1965); *J. Org. Chem.*, **31**, 2284 (1966).

(5) S. Udenfriend, H. Weissbach, and C. T. Clark, *J. Biol. Chem.*, **215**, 337, (1955).

(6) H. Jensen and K. K. Chen, *ibid.*, **116**, 87 (1936).

(7) H. Wieland and T. Wieland, *Ann.*, **528**, 234 (1937).

(8) V. Erspamer, *Nature*, **170**, 281 (1952).

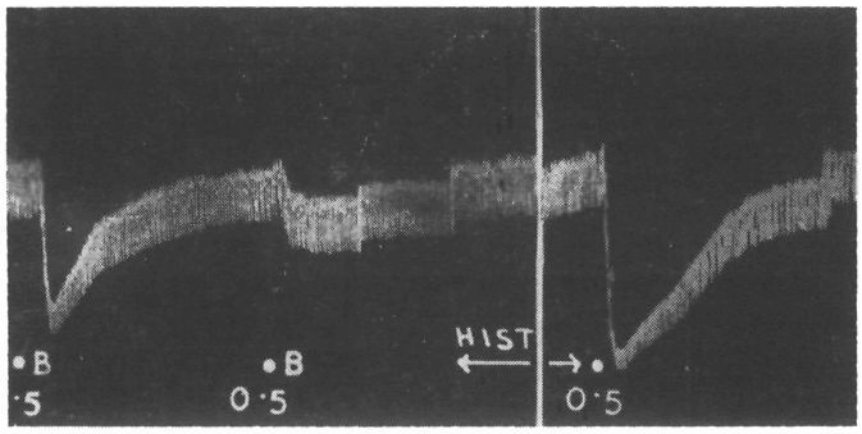

Figure 3.—Effect of bufotenidine on the carotid blood pressure of the anesthetized dog. At the white line the drum was stopped for 30 min. Hypotensive response was reproduced after histamine (5 μg/ml/min) perfusion for 30 min.

spasm in isolated frog rectus abdominis muscle. It did not alter the KCl-induced spasm. This blocking effect was compared against *d*-tubocurarine, and ED_{50} were calculated by drawing regression curves. The ED_{50} values of the alkaloid and *d*-tubocurarine were determined as 1.4 and 1.05 μg/ml, respectively.

In vivo, when administered to albino mice and rats in doses of 10 mg/kg ip and *po*, respectively, the alkaloid caused the death of all five mice and ataxia in all ten rats. The death was due to respiratory failure, evident by deep cyanosis and because the heart was still beating when respiration had ceased. There was no death or ataxia when the alkaloid was administered to mice in doses of 2 mg/kg ip. The alkaloid produced head drop followed by death in rabbits in a 5–6-mg/kg iv dose administered against 0.3–0.5 mg/kg iv of *d*-tubocurarine. The ED_{50} could not be determined due to paucity of material. These studies suggest that the alkaloid is a potent neuromuscular blocking agent. Though its ED_{50} *in vitro* was not significantly lower than that of *d*-tubocurarine, the latter was found to be at least eight to twelve times more potent *in vivo* experiments. Further study is needed to resolve this discrepancy in the results of *in vivo* and *in vitro* experiments.

Effect on Smooth Muscles.—The isolated loop of guinea pig and dog intestine *in situ*, the isolated uterus of albino rats and guinea pigs, and the dog's tracheal chain were employed to study the effect of the alkaloid on smooth muscles.

Intestine.—*In situ* experiments with the alkaloid produced spasm and repeated doses resulted in tachyphylaxis. This was thought to be due to histamine release. In an isolated loop of intestine the alkaloid relaxed the muscle in concentrations of 1–10 μg/ml. In higher concentrations, above 20 μg/ml, the alkaloid antagonized the spasmodic effects of histamine, AcCh, and $BaCl_2$. The spasmolytic action, therefore, is nonspecific in character.

Uterus.—The drug produced its own spasm, more consistently, in isolated guinea pig uterus than in isolated albino rat uterus. This spasm was only seen up to a concentration of 20 and 10 μg/ml in guinea pig and albino rat uterus, respectively. There was no tachyphylaxis. In higher concentrations the drug blocked AcCh-induced spasm. It is possible that the basis of use of the plant extract (rhizomes) in indigenous systems of medicine[1] "as a stimulant of menstrual discharge" was due to the stimulant action of the alkaloid.

Dog's Tracheal Chain.—The alkaloid (up to 80 μg/ml) had no effect of its own nor did it modify the AcCh-induced (1 μg/ml) spasm.

Experimental Section[9]

The general procedure for the separation and identification of the alkaloids involved column chromatography over Brockmann alumina and paper and thin layer chromatography of the individual components in the presence of marker compounds; determination of uv absorption spectra; and preparation of picrate, HCl, HI, and MeI salts where possible. Descending paper chromatography was used on Whatman No. 1 paper using *n*-BuOAc–*n*-BuOH–AcOH–H_2O (85:15:40:22). In the tlc, silica gel G was used as the adsorbant and MeOH as the developer. The indolic bases showed pale yellow to yellow-green fluorescence under uv light on paper and did not respond to the Jepson and Steven's test[10] for N_b-unsubstituted tryptamines indicating thereby that the basic N in these compounds is substituted.

Isolation of the Alkaloids.—Dried and milled rhizomes (700 g) were extracted (95% EtOH), in a percolator, at room temperature, for 4 weeks. The EtOH extract was concentrated under reduced pressure to give a brown viscous consistency (112 g) which was poured into 2% AcOH (100 ml) with stirring, and the mixture was kept overnight at ordinary temperature. Suspended impurities were filtered off and the filtrate was shaken with $CHCl_3$ (three 50-ml portions) which removed the $CHCl_3$-soluble acetates (10.3 g, fraction A). The pH of the aqueous solution was brought to 9 with NH_3 and the liberated bases were extracted ($CHCl_3$, three 50-ml portions). The polymeric material remained insoluble in $CHCl_3$ and was filtered, washed (H_2O), and dried (4.1 g), mp 80 and 235–240°; λ_{max} 300–305 mμ (sh); λ_{max} (Nujol) 2.95 μ (NH); R_f (tlc) 0.0; N, 4.82 and 5.1%.

The $CHCl_3$ solution containing the strong bases was washed (H_2O) and dried, and the solvent was removed under reduced pressure when a brown basic gum (3.7 g, fraction B) was obtained. The aqueous alkaline mother liquor was treated with a saturated aqueous solution of ammonium reineckate and the light pink reineckate complex was kept for subsequent regeneration of water-soluble strong bases (fraction C).

Fraction A. $CHCl_3$-Soluble Acetates.—The residue from this fraction was taken in PhH and the PhH solution was extracted with 0.1 *M* aqueous citric acid (100 ml). The aqueous acidic layer was basified (NH_3), the liberated bases were extracted ($CHCl_3$), and the solvent was removed from the organic layer when a thick brown oil (0.04 g) was obtained.

N,N-Dimethyltryptamine.—The identity of the above base, R_f 0.74, was established by cochromatography with authentic N,N-dimethyltryptamine [R_f 0.74; uv, λ_{max} 222 mμ (log ε 4.48), 277 (3.77), and 288 (3.75)] and from the formation of a picrate from EtOH; mp 168° undepressed on admixture with the authentic N,N-dimethyltryptamine picrate, mp 168°.

Fraction B. $CHCl_3$-Soluble Strong Bases.—The total basic gum (3.7 g) was dissolved in $CHCl_3$ (10 ml) and chromatographed on partially deactivated Brockmann alumina (35 × 4 cm). Elution was carried out with 100-ml portions of petroleum ether, (bp 40–60°), petroleum ether–PhH (90:10, 80:20, 50:50), PhH, PhH–$CHCl_3$ (95:5, 90:10, 80:20, 50:50, 25:75), $CHCl_3$–MeOH (99:1, 98:2, 95:5), and MeOH.

5-Methoxy-N-methyltryptamine.—The $CHCl_3$–PhH (75:25) eluates were combined and concentrated under reduced pressure. The residue (0.016 g), R_f 0.58, was cochromatographed with authentic 5-methoxy-N-methyltryptamine, R_f 0.58, which showed a single spot at the expected R_f; uv, λ_{max} 224 mμ (log ε 4.53) and 288 (3.82).

The base formed a red picrate from MeOH; mp 220°, mmp 222° with authentic 5-methoxy-N-methyltryptamine picrate, remaining undepressed.

Bufotenine.—The $CHCl_3$–MeOH (99:1) eluates were combined and the solvent was removed under reduced pressure when a brown amorphous mass (0.18 g) was obtained, R_f 0.27; cochromatography with authentic bufotenine showed R_f 0.27; uv, λ_{max} 224 mμ (log ε 4.46), 278 (3.93), 294 (3.90), and 305 (3.52).

(9) All melting points were taken in open capillary and are uncorrected. Uv spectra were recorded in a Carl Zeiss Universal spectrophotometer, VSU1, using spectral EtOH. Microanalyses were performed by Dr. A. Bernhardt, Max Plank Institute, Mulheim (Ruhr), Germany.

(10) J. B. Jepson and B. J. Stevens, *Nature*, **172**, 772 (1953).

The base afforded a yellow picrate from EtOH; mp 178°, mmp 177–178° with the authentic bufotenine dipicrate, remaining undepressed. The N_b-oxide was crystallized from Me_2CO–EtOH as rods, mp 217° (lit.[11] mp 217°).

Bufotenidine.—MeOH washings of the alumina column on evaporation left a brown basic gum (2.3 g), R_f 0.18 (as the major area of intensity with both Dragendorff and Ehrlich reagents) plus three other spots, R_f 0.0, 0.08, and 0.22. The major component was separated by preparative tlc over silica gel G using MeOH as the developer; R_f 0.16; uv, λ_{max} 218–220 and 284–288 mμ; red picrate from aqueous EtOH; mp 198–200°, mmp 198–200° (lit.[7] mp 198°) with authentic bufotenidine picrate, remaining undepressed.

A portion of the basic gum was directly converted to the picrate. The picrate was crystallized from aqueous EtOH as red needles, mp 198°. *Anal.* Calcd for $C_{13}H_{18}N_2O \cdot C_6H_3N_3O_7$: N, 15.67. Found: N, 15.82.

The base hydriodide crystallized from EtOH as light gray needles, mp 209–210°, mmp 209–210° with bufotenine methiodide, remaining undepressed. *Anal.* Calcd for $C_{13}H_{18}N_2O \cdot HI$: N, 8.09. Found: N, 7.93.

Fraction C. Water-Soluble Bases.—The pink reineckate complex, mp 168–170° dec, was dissolved in Me_2CO and passed through a column of De-acidite FF (pH 8).[12] The EtOH eluates on evaporation yielded a crystalline alkaloid (0.44 g), R_f 0.06 (plus two other minor components, R_f 0.16 and 0.31).

Dehydrobufotenine.—The crystalline compound, obtained above, had a double melting point, 202 and 217°, R_f 0.06 (paper) and 0.04 (tlc). The compound was identified as dehydrobufotenine,[6,7] mp 198 and 218°, on the basis of its physical and chemical properties and those of its salts; uv, λ_{max} 218–220 mμ (log ε 4.55) and 285 (3.98); violet color with α-nitroso-β-naphthol reagent;[5] and negative Jepson and Stevens test[10] for N_b-unsubstituted tryptamines. *Anal.* Calcd for $C_{12}H_{14}N_2O \cdot H_2O$: C, 65.45; H, 7.27; N, 12.72. Found: C, 64.98; H, 6.99; N, 12.81.

The HCl salt crystallized from EtOH as needles, mp 237–238° (lit.[7] mp 237–238°), and the HI salt crystallized from aqueous EtOH as light gray rods, mp 243–245° (lit.[7] mp 243–245°).

The picrate crystallized from aqueous EtOH as yellow needles, mp 182–184° (lit.[7] mp 183–184°). *Anal.* Calcd for $C_{12}H_{14}N_2O \cdot C_6H_3N_3O_7$: N, 16.27. Found: N, 15.98.

Acknowledgment.—The authors are grateful to the C.S.I.R., New Delhi, for awarding a Junior Research Fellowship to one of them (S. K. D.).

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The Reaction of Benzylpenicillenic Acid with Thiol-Containing Compounds. The Formation of a Possible Penicillin Antigenic Determinant[1]

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It has been shown that the penicilloyl determinant in penicillin allergy can be formed by the reaction of the penicillin degradation product, benzylpenicillenic acid, with free amino groups in proteins. This study was conducted to determine whether benzylpenicillenic acid could react similarly with another functional group in proteins, the SH group. The reactivity of benzylpenicillenic acid at pH 7.5 was measured spectrophotometrically and was found to be ten times greater with compounds having free sulfhydryl groups than those having free amino groups. Experiments using compounds with missing or blocked SH groups indicated the sulfhydryl group was the reactive species and suggested the possible formation of a product different from those reported previously. Tlc of the products of the reaction of benzylpenicillenic acid and ethanethiol at pH 7.5 disclosed the presence of four components. Characterization of these components by nmr and mass spectrometry revealed that they are stereoisomers of the thiol ester of benzylpenicilloic acid, α-ethylthiobenzylpenicilloate. These results indicate that benzylpenicillenic acid can react with free SH groups of protein to form thioesters in much the same way it reacts with free amino groups to form amides.

In allergenic responses to simple compounds it is known that irreversible binding to protein is necessary for the simple chemical compound to sensitize and elicit an allergic reaction. Benzylpenicillenic acid, which forms spontaneously from benzylpenicillin,[2a] can react irreversibly with proteins[2b] and is believed to play a role in penicillin allergy.[3,4]

It has been demonstrated that penicilloyl compounds may be formed by reaction of either penicillenic acid[5,6] or by direct interaction of penicillin[7–9] with free amino groups of protein. The penicilloyl group is believed to be the major antigenic determinant in penicillin allergy.[10,11]

The present work is a study of the reactivity of benzylpenicillenic acid with another functional group found in proteins, the sulfhydryl group. Cysteine, N-acetylcysteine, serine, penicillamine, homocysteine, methionine, glutathione, ε-aminocaproic acid, 2-mercaptoethanol, and ethanethiol serve as model compounds for free amino groups and free as well as blocked SH groups of proteins. The reactivity of penicillin and penicillenic acid with these model compounds is compared, the implication being that penicillenic acid reacts with free SH groups of proteins to form thioesters in much the same way it reacts with free amino groups to form amides. The possibility that the product of this reaction, a penicilloyl thioester, may be a determinant of penicillin allergy is discussed.

(1) Presented in part at the 155th National Meeting of the American Chemical Society, San Francisco, Calif., 1968.

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