THE ACTION OF ACETIC ANHYDRIDE ON N-METHYLTRYPTOPHANS

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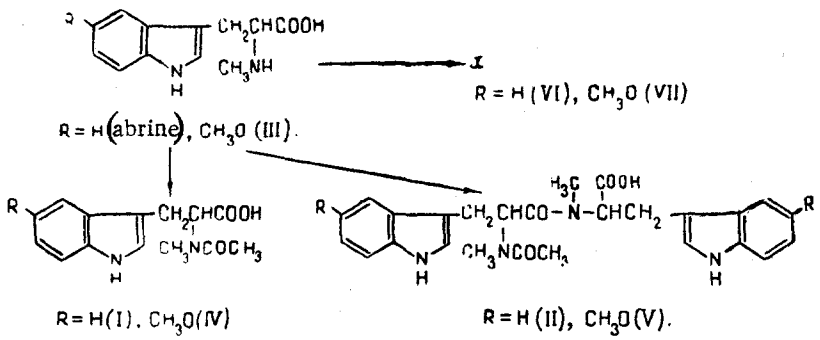
Of the N-methylated amino acids, only sarcosine has been studied relatively well, although in nature other compounds of this type are found. In particular, abrine (N-methyltryptophan) has been obtained from the seeds of the plant Abrus precatorius [1, 2]. The N-methyl derivatives of isoleucine, leucine, and valine are present in the antibiotics of the enniatine group [3].

In a study of the ergot alkaloids with respect to their biosynthesis, we have synthesized dl-abrine and the previously unreported 5-methoxyabrine [4], these amino acids being found to have unusual behavior in their reaction with acetic anhydride. It is known that α-amino acids form acetyl derivatives of oxazolones when heated with acetic anhydride [5].

Thus, acetyltryptophan is readily obtained by the action of acetic anhydride on a solution of tryptophan in glacial acetic acid [6]. However, on passing to the corresponding N-methylamino acid, dl-abrine, the reaction becomes more complicated. Cahill and Jackson [7], by acetylating dl-abrine with a gentle current of ketene, obtained acetylabrine with mp 171° C. Later, Uhle and Harris [8] synthesized acetylabrine by the action of acetic anhydride in alkaline solution at 0° C and isolated it in the form of the monohydrate which melted at 80°-82° C. We have acetylated dl-abrine by various methods: acetic anhydride in glacial acetic acid at 50°-60° C and room temperature, glacial acetic acid at the boil, and acetic anhydride in alkaline solution in the cold. On boiling with glacial acetic acid, dl-abrine was recovered unchanged. In the other experiments, a mixture of substances melting at about 100° C and consisting of the acetylamino acid (I) and the acetyldipeptide (II) were formed. This can be seen from the results of electrophoresis and paper chromatography. Descending chromatography on Leningrad type B paper was used (Table 1); the mixture was separated in the isopropyl alcohol-ammonia (10 : 1) system. Acetylabrine (I) and acetylabrylabrine (II) posess almost identical solubilities in water and alcohol, and they cannot be separated by crystallization.



However, by carrying out acetylation at 30°-35° C we were able to obtain acetylabrine (I) not contaminated with dipeptide (mp 80°-81° C). But if the reaction is carried out at 60°-70° C, the acetyl dipeptide (II) will predominate in the reaction mixture, and under these circumstances it was isolated and purified. This substance is soluble in alkali, hydrolyzing to form abrine, and its electrophoretic mobility is half that of acetylabrine.

The formation of acetyl dipeptides under the conditions mentioned has not been described previously and we therefore carried out corresponding experiments with 5-methoxy-dl-abrine (III). The action of acetic anhydride on a solution of the amino acid (III) in glacial acetic acid gave not only the acetyl derivative (IV) but also 5-methoxyacetylabryl-5-methoxyabrine (V) with a yield of 48% . The acetyl dipeptide (V) obtained is soluble in alkali, is hydrolyzed by 55% phosphoric acid with the formation of the original amino acid, and its electrophoretic mobility is half that of 5-methoxy-acetylabrine (IV).

If unmethylated typtophan is heated with acetic anhydride in the absence of acetic acid, it rapidly resinifies and only acetyltrptophan and the original amino acid can be identified in the reaction mixture by paper chromatography. Under analogous conditions, abrine and 5-methoxyabrine (III) were converted into new compounds which were purified by chromatography on alumina. The individuality of these substances was confirmed in a thin layer of alumina and a mixture of silica gel and gypsum (Table 2).

Table 1

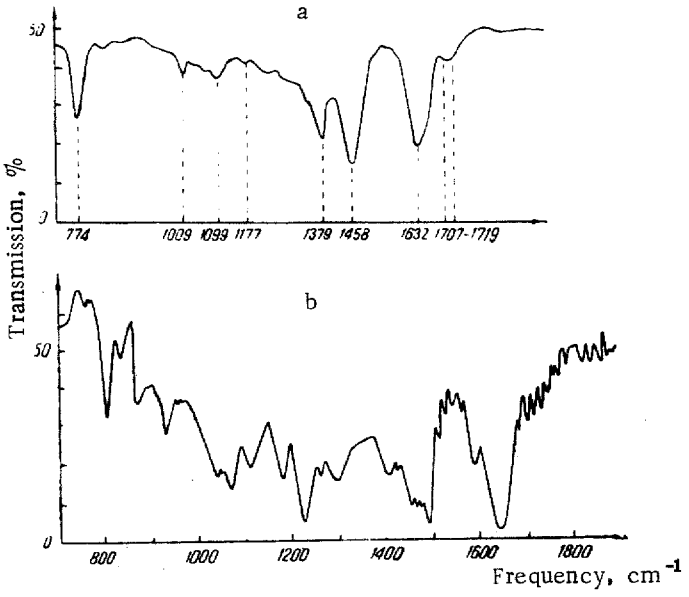
Paper Chromatography of the Products of the Acetylation of Abrine and 5-Methoxyabrine

Substance	Butanol–acetic acid–water (4:1:5)	Isopropanol–ammonia–water (10:1:1)	Coloration with Ehrlich's reagent
5-Methoxyabrine	0.52	0.30	Blue
5-Methoxyacetylabrine	0.89	0.47	
Acetyl-(5-methoxyabryl)-5-methoxyabrine	0.94	0.62	Purple
Abrine	0.58	0.35	
	0.90	0.53	
Product of the acetylation of abrine	0.96	0.66	
Acetylabrylabrine	0.95	0.66	

It is natural to assume that the products of the reaction of abrine and 5-methoxyabrine with pure acetic anhydride [substances (VI) and (VII)] are compounds of the peptide type. In particular, we assumed that these substances were cyclopeptides [9]. In actual fact, when both substances were hydrolyzed with 6 N hydrochloric acid for 10 hr and with 70% phosphoric acid for 2 hr, the original amino acids were detected. For this purpose, the hydrolyzates were chromatographed on paper in the butanol–acetic acid–water (4:1:5) and the isopropanol–ammonia–water (10:1:1) systems.

The compounds under investigation proved to be very stable to alkaline hydrolysis; no amino acids were found after boiling for 30 hr with 1 N sodium hydroxide in methanol. Both the polymeric substances were insoluble in dilute mineral acids and alkalis. An electrophoretic investigation showed that they were stationary in an electric field both in an alkaline system (pH 8.9) and in an acid system (30% acetic acid). These facts, and also the mobility of both substances on neutral alumina, show the absence of free terminal amino and carboxyl groups from their molecules. Moreover, the intensive evolution of carbon dioxide during the reaction showed the elimination of the COOH group.

The IR spectra of substances (VI) and (VII) (figure) show strong bands with frequencies of 1632 and 1635 cm^{-1} corresponding to the stretching vibrations of a carbonyl group in an amide [the IR spectrum of compound (VI) was taken on a IKS-14 instrument, and that of compound (VII) on a UR-10 instrument]. As was to be expected, the bands due to the NH deformation vibrations in the 1650–1550 cm^{-1} region are not shown, since the compounds were obtained from N-substituted amino acids. In this respect, the IR spectra of the compounds investigated are similar to the IR spectra of poly-



IR spectra of a) compound (VI) (paraffin oil) and b) compound (VIII) (KBr).

Table 2

Chromatography of the Products of the Reaction of Abrine and 5-Methoxyabrine with Acetic Anhydride in a Thin Layer of Silica Gel and Gypsum

Substance	Cyclohexane–ethyl acetate (1:1)	cyclohexane–ethyl acetate (1:2)	Benzene–acetone (1:1)	By Matthias' method: cyclohexane–ethyl acetate (2:5)	By Matthias' method: Benzene–acetone (1:1)
(VI)	diffuse	0.20 diffuse	0.52	0.30	0.46
(VII)	—	0.30 "	0.55	0.43	0.52

peptides containing no NH bond. Thus, polyproline absorbs only at 1640 cm^{-1}, and polysarcosine at 1600 cm^{-1} [10]. In addition to this, the spectrum of compound (VI) has absorption at 1707–1719 cm^{-1} (figure, a) corresponding to the stretching vibrations of a keto group, although with a considerably lower intensity than the peaks of the amide carbonyls. The spectrum of compound (VII) also has some small peaks in this region (figure, b).

Apparently, during the synthesis of substances (VI) and (VII), in addition to peptide formation, ketonization takes place in the manner of a Dakin-West reaction [11]; however, the results obtained do not enable an unambiguous structure to be assigned to substances (VI) and (VII). A more detailed study of their structure will be the object of subsequent communications.

Experimental

Acetylabrine (I). With stirring, a solution of 1 ml of acetic anhydride in 5 ml of glacial acetic acid was added in drops to a solution of 1 g of abrine in 20 ml of glacial acetic acid. The mixture was kept for 2 hr at room temperature and was then heated for half an hour at 30°–35° C and left for 20 hr. The reaction mixture was evaporated in vacuum, and the residue was treated with 20 ml of water and one drop of concentrated hydrochloric acid, heated to 50° C, and cooled. The light brown oil which separated out was removed by decantation and dried in a vacuum desiccator. Yield 0.2 g. The solution was evaporated to 1/3 and extracted twice with ethyl acetate. The ethyl acetate was evaporated to dryness, and 0.3 g of a solid colorless residue of acetylabrine, mp 80°–81° C [8] was obtained. This sample was pure and identical with the first portion, and had R_f 0.74 in a thin layer of a mixture of silica gel and gypsum [n-propanol–ammonia (7 : 3) system, spots revealed with Ehrlich's reagent]. The acid solution remaining after the ethyl acetate extraction was neutralized with ammonia, and the precipitate which was deposited was separated off. This gave 0.32 g of the initial abrine. The total yield of acetylabrine monohydrate was 0.5 g (57.5%), calculated on the abrine that had reacted. The electrophoretic mobility was 8.9 cm. Here and below electrophoretic mobilities were determined in a buffer system with pH 8.6 (19.07 g/*l* of borax + 10 g/*l* of boric acid), 6 hr at a voltage of 300 V.

Acetylabrylabrine (II). With stirring, a solution of 1.02 g of acetic anhydride in 20 ml of glacial acetic acid was added in drops to a solution of 2.18 g of abrine in 25 ml of glacial acetic acid heated to 60°–70° C. Heating and stirring were continued for another 30 min, and then the acetic anhydride and the acetic acid were distilled off in vacuum, the residue was dissolved in 5% sodium hydroxide solution, and the solution was acidified to congo red with dilute hydrochloric acid. The amorphous powder that deposited was separated off, washed with water, and dried in a vacuum desiccator. This gave 0.44 g of acetylabrylabrine (II), slightly contaminated with acetylabrine. The filtrate was evaporated to small bulk in vacuum and neutralized to pH 6 with sodium hydroxide, 0.52 g of unchanged abrine being recovered. For analysis, substance (II) was dissolved in 2% sodium hydroxide, and the solution was filtered, washed with ether, and acidified with 2 N hydrochloric acid after the ether residues had been eliminated by heating; the sample melted diffusely in the range 140°–160° C. Yield 24%, calculated on the abrine that had reacted. Electrophoretic mobility 4.1 cm.

Found, %: C 65.51, 65.24; H 6.03, H 6.51; CH_3CO 9.65; 9.51; mol. wt. 488 (Rast); 435 (titration). Calculated for $C_{26}H_{28}N_4O_4 \cdot H_2O$, %: C 65.27; H 6.31; CH_3CO 9.00; mol. wt. 478.5.

5-Methoxyacetylabrine (IV) and acetyl-(5-methoxyabryl)-5-methoxyabrine (V). With stirring, a solution of 3 ml of acetic anhydride in 15 ml of glacial acetic acid was added to a heated solution of 3 g of 5-methoxyabrine (III) [4] in 30 ml of glacial acetic acid. Heating (50°–60° C) and stirring were continued for a further 30 min. The bulk of the acetic acid was distilled off in vacuum, the residue was poured into water, and the mixture was left to stand overnight. The brown oil that had separated was removed by decantation, and a few drops of dilute hydrochloric acid were added to the decanted clear solution and it was left in the refrigerator. After some days, colorless crystals (0.4 g) of 5-methoxyacetylabrine (IV) with mp 165°–170° C deposited. The oil was dissolved in alkali and precipitated with

hydrochloric acid. The resulting light brown substance (3 g) consisted of a mixture of compounds (IV) and (V). It was dissolved in alcohol, water was added to the solution to turbidity, and it was cooled. The oil which separated out at first was decanted off, and then colorless crystals of 5-methoxyacetylabrine were deposited from the transparent solution. These separations were repeated until it had been isolated completely from the oil. The total yield was 1.75 g (50%) of 5-methoxyacetylabrine (IV) with mp 165°–170° C (aqueous alcohol).

Found, %: 62.14, 62.07; H 6.21, 6.15. Calculated for $C_{15}H_{18}N_2O_4$, %: C 62.10; H 6.24.

The remaining noncrystallizing oil was dissolved in dilute sodium hydroxide solution, and the solution was washed with benzene and then with ether. The alkaline solution was filtered and was carefully acidified with 2 N hydrochloric acid. The amorphous powder of compound (V) was separated on a low permeability filter, washed with water, and dried. Yield 1.5 g (48%), mp 143°–153° C (decomp). The electrophoretic mobility of compound (IV) was 7.8 cm and that of compound (V) 3.6 cm.

Found, %: C 64.52, 64.61; H 6.26, 6.21; N 11.36; CH_3CO 7.2, 7.1; mol. wt. 596 (Rast); 506, 532 (titration). Calculated for $C_{28}H_{32}N_4O_6$, %: C 64.60; H 6.20; N 10.76; CH_3CO 8.06; mol. wt. 520.5.

Reaction of abrine with acetic anhydride. A mixture of 2 g of dl-abrine and 10 ml of acetic anhydride was boiled for 1 hr. The reaction mixture was poured into 50 ml of water, well stirred, and left overnight at room temperature. The brown oil solidified almost completely. It was triturated until it had been converted into a yellow powder and then 30 ml of water was added; the powder was filtered off and washed with water. The yield of the substance after drying at 100° C was 1.81 g. The filtrate was evaporated to dryness in vacuum; the residue (0.2 g) consisted of at least three substances (chromatography in a thin layer of alumina) which were not investigated further. The substance obtained was dissolved in dioxane and was absorbed on a column of alumina (16.5 × 1.4 cm). Substance (VI) was eluted with dioxane. The dioxane was evaporated in vacuum giving a residue in the form of a foamy glassy mass weighing 1.45 g. For analysis, part of the substance was dissolved in the minimum amount of dioxane and was precipitated with ether. The pale yellow precipitate of substance (VI) that was deposited was separated off, carefully washed with ether, and dried in vacuum at 100° C, mp 175°–185° C (decomp., darkens at 166° C).

Found, %: C 71.37; H 6.65; N 13.14.

Reaction of 5-methoxyabrine (III) with acetic anhydride. 5-Methoxyabrine (2.5 g) was boiled with acetic anhydride for 15 min. The reaction mixture was poured into 100 ml of cold water and left overnight. The brown oil that separated out solidified as the acetic anhydride decomposed. The solid precipitate was separated off, washed with water, and dried. Yield 2.4 g. On chromatography in a thin layer of alumina, this substance gave two spots with R_{f_1} 0.00 and R_{f_2} 0.75 [benzene–ethanol (5:1)], the second being the main one. For purification, 0.5 g of the substance was dissolved in ethyl acetate and adsorbed on a column of alumina. It was eluted with ethyl acetate, the solvent was evaporated to dryness in vacuum, and the residue was dissolved in ethyl acetate and precipitated with ether. The yellow powder which was deposited was separated off and carefully washed with ether. This gave 0.3 g of the pure substance (VII) with mp 190°–205° C (darkening at 175° C).

Found, %: C 66.81, 66.70; H 7.14, 6.95; N 11.31, 11.48.

Summary

Depending on the reaction conditions, acetylation of the methylamino acid dl-abrine and 5-methoxy-dl-abrine gives acetylamino acids, acetyl dipeptides, and more complex compounds of the peptide type.

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