STRUCTURES OF THE PRODUCTS OF THE REACTION OF PENILLOIC ACIDS WITH ACID ANHYDRIDES

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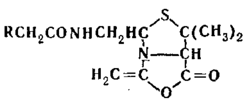
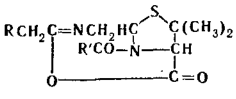
N^4-Acylpenilloic acid lactam structures were assigned to the products of the reaction of penilloic acids with acid anhydrides on the basis of a chemical and physicochemical study.

Among the important products of the cleavage of penicillins are penilloic acids, which are obtained both by decarboxylation of penicilloic acids and directly from penicillins by heating aqueous solutions of them; they are also formed during the cleavage of a number of isomers of penicillins. Although penilloic acids have been studied in detail with respect to their chemistry, some peculiarities of their chemical behavior have still not been investigated sufficiently, and this complicates the interpretation of individual transformations of the penicillins. This is particularly the case with respect to the study of their acylation products.

During the period of intensive study of penicillins, the question of their reaction with acetic acid and acetic anhydride was subjected to a special investigation [1], but the structures of the compounds obtained during this investigation, particularly their neutral acylation products, were not ascertained. In 1957, during a study of the reaction of benzyl- and phenoxymethylpenilloic acids with acetic anhydride, neutral products were obtained which, with respect to their overall formula, differed from acetylpenilloic acids by the loss of the elements of water. Structural formula I was assigned to these products on the basis of their elementary composition, their color reaction with tetranitromethane, and several transformations [2].



I



II

However, subsequent chemical and physicochemical investigations (PMR, IR, UV, and mass spectroscopy) indicated that this formula is incorrect. Thus, the IR spectra of the products of the acylation of penilloic acids (in mineral oil pastes, in pellets, and in chloroform solution) did not contain bands which correspond to the valence and deformation vibrations of the amide group; the PMR spectra do not contain peaks for hydrogens attached to a double bond; in the mass spectrum of the product of the reaction of benzylpenilloic acid with acetic anhydride the ion peak (a) with m/e 118 is the most intense peak (which does not agree with formula I); etc. It therefore became necessary to establish the correct structures of the neutral products of the acylation of penilloic acids.

We synthesized the products of the reaction of benzyl- and phenoxymethylpenilloic acids with acetic, propionic, and butyric anhydrides (see Scheme 1). A number of similar compounds (their properties are given in Table 1) were obtained, which indicates the general character of this reaction. As a result of an investigation of these compounds several formulas were proposed for them, of which II and III seemed most probable. The choice between them was made on the basis of mass spectrometric data which undoubtedly

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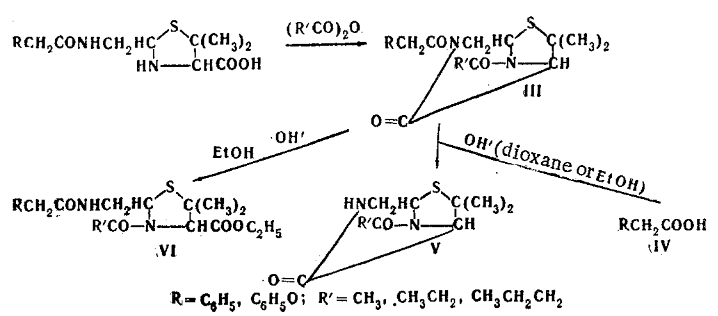
TABLE 1. Properties of N^4-Acylpenilloic Acid Lactams

Structure III		mp, °C	Empirical formula	Found					Calculated					UV spectrum (in ethanol)		Yield, %
R	R′			C, %	H, %	N, %	S, %	M*	C, %	H, %	N, %	S, %	M	λ_{max}, nm	ε	
C_6H_5	CH_3	134—136,5 †	$C_{17}H_{20}N_2O_3S$	61,55	6,18	8,28	9,67	332	61,42	6,06	8,43	9,64	332,42	210—210,5	19 940	44
C_6H_5O	CH_3	195—197 ‡	$C_{17}H_{20}N_2O_4S$					348					348,62	217	17 420	30
C_6H_5	CH_3CH_2	128—129	$C_{18}H_{22}N_2O_3S$	62,48	6,34	8,06	9,28	346	62,40	6,40	8,08	9,26	346,44	210—210,5	21 600	30
C_6H_5O	CH_3CH_2	134—135,5	$C_{18}H_{22}N_2O_4S$	59,51	6,11	7,74	8,74	362	59,64	6,12	7,72	8,85	362,44	217	20 600	20
C_6H_5	$CH_3CH_2CH_2$	107—108	$C_{19}H_{24}N_2O_3S$	63,08	6,69	7,86	8,85	360	63,30	6,71	7,77	8,89	360,47	210—210,5	18 300	28

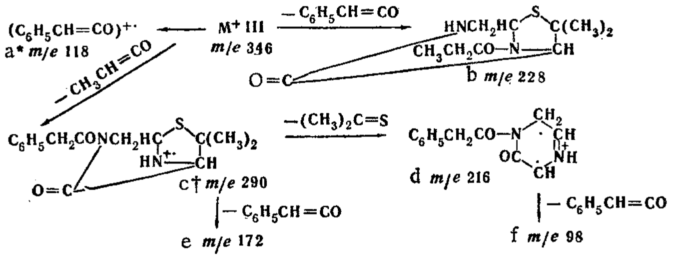
* From mass spectrometry.
† mp 121-123° [2].
‡ mp 197-198° [2].

Scheme 1



constitute evidence in favor of formula III. An intense molecular ion peak is present in the mass spectrum of the product of the reaction of benzylpenilloic acid with propionic anhydride (Fig. 1). The remaining peaks, except for the peaks with m/e 91 $(C_7H_7^+)$ and 118, which are typical for benzylpenicillin derivatives [3, 4], have low intensities. This indicates that the compound has a stable cyclic structure. The formation of a fragment with $(M-56)^+$ due to the elimination of a molecule of methyl ketone is characteristic for N-propionyl derivatives of heterocycles [5]. The further ejection of 74 mass units (the thioacetone molecule) is typical for penicillins [3] and their isomers which contain a thiazolidine ring. An ion with an m/e value which is 118 mass units less (elimination of a phenylketone molecule) corresponds to each of the ions enumerated above. This indicates that the $C_6H_5CH_2CO$ group is directly bonded to the cyclic system. Diacylamide formula III can be assigned to the investigated compound on the basis of the above. The paths for fragmentation of III are presented in Scheme 2.

Scheme 2



The products of the reaction of benzyl- and phenoxymethylpenilloic acids with acetic and butyric anhydrides give the same fragmentation pattern. An $(M-42)^+$ ion (elimination of a ketene molecule) is formed in the spectra of compounds obtained by the action of acetic anhydride. In the spectrum of the product obtained by the action of butyric anhydride, the elimination of an ethylketene molecule results in an $(M-70)^+$ ion. The spectra of phenoxymethylpenilloic acid derivatives contain peaks shifted by 93 mass units due

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*Ion a can also form from c and d.

† The elimination of a propionyl residue can also occur without the migration of a hydrogen atom. In this case, fragment c′ with m/e 289 develops and decomposes via a similar scheme to give ions d′-f′ which differ from corresponding ions d-f by 1 mass unit.

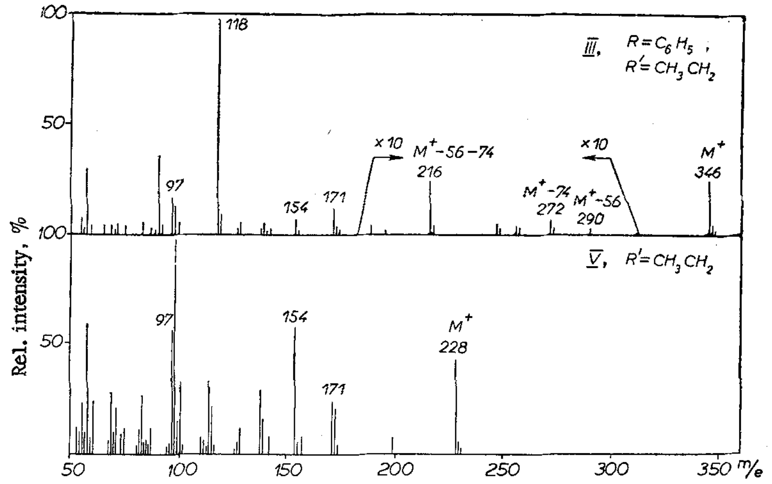

Fig. 1. Mass spectra of the lactams.

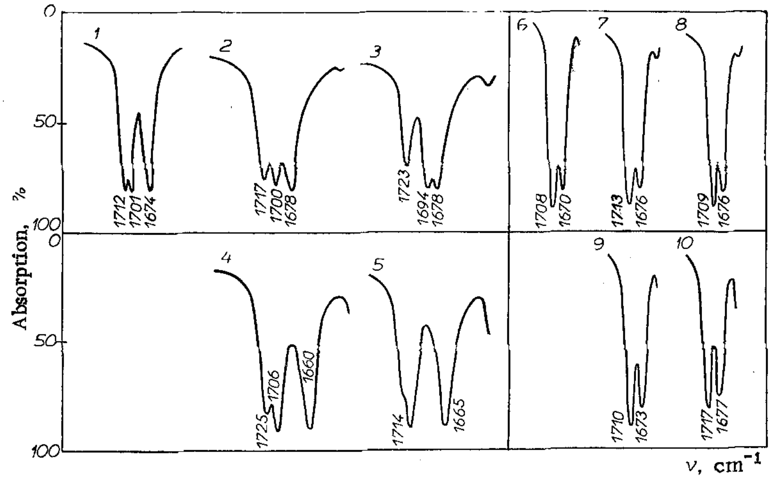

Fig. 2. IR spectra of N^4-acylpenilloic acid lactams (III): 1, 6) R = C_6H_5, R' = $CH_3CH_2CH_2$; 2,7) R = C_6H_5, R' = CH_3CH_2; 3, 8) R = C_6H_5, R' = CH_3; 4,9) R = C_6H_5O, R' = CH_3CH_2; 5,10) R = C_6H_5O, R' = CH_3. 1-5) Mineral oil pastes; 6-10) $CHCl_3$ solutions (1 = 0.267).

to elimination of a C_6H_5O radical in addition to the ion peak (M^+) and peaks corresponding to c and d; this is in agreement with data on the fragmentation of phenoxymethylpenicillin isomers. This makes it possible to conclude that N^4-acylpenilloic acid lactam structure III can also be assigned to all of the indicated compounds.

On the other hand, formula II contradicts the mass spectrometric data since the formation of ion a with m/e 118 (the maximum peak in the spectrum) from the molecular ion should be associated with the simultaneous breaking of three bonds at one atom, which is extremely unlikely.

The conclusion drawn on the basis of the mass spectrometric investigation of the compounds obtained is not contradicted by their IR spectra, which contain three intense, partially overlapped bands at 1665-1730 cm^{-1}, the intensities of which indicate that they belong to a carbonyl absorption. A comparison of the IR spectra (Fig. 2) of the neutral products of the acylation of benzyl- and phenoxymethylpenilloic acids by the homologous fatty acid anhydrides indicated the presence of an absorption band shift from 1694 to 1701 cm^{-1} and from 1714 to 1725 cm^{-1}, respectively, for derivatives of benzyl- and phenoxymethylpenilloic acids. On the basis of this, this absorption was assigned to the vibration of the tertiary amide of the thiazolidine ring. The other two intense bands are apparently due to the vibrations of the carbonyl group

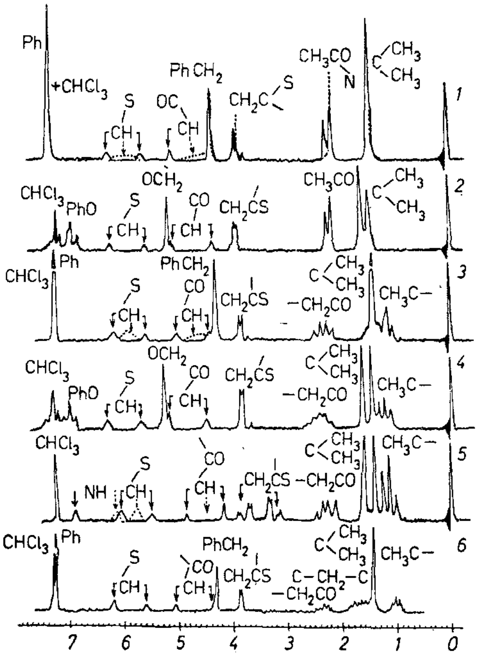

Fig. 3. PMR spectra: 5) lactam V (R' = CH_3CH_2) and N^4-acylpenilloic acid lactams (III): 1) R = C_6H_5, R' = CH_3; 2) R = C_6H_5O, R' = CH_3; 3) R = C_6H_5, R' = CH_3CH_2; 4) R = C_6H_5O, R' = CH_3CH_2; 6) R = C_6H_5, R' = $CH_3CH_2CH_2$. (The dashed lines in spectra 1, 3, and 5 indicate the most intense changes which occur during heating.)

of the diacylamide grouping.* The presence of an absorption at about 1200 cm^{-1}, which may be due to the vibration of the C−N bond, does not contradict this assignment. The elevated intensity of the high-frequency band observed in the IR spectra in $CHCl_3$ solution is apparently explained by superimposition on the high-frequency component of the vibration of the carbonyl group of the tertiary amide, which has a higher frequency in solution than in the solid state.

Everything stated above is confirmed by the PMR spectra of the compounds, where peaks are seen which are common to all of the compounds and are readily assignable to groupings common to all of them (Fig. 3). The peaks which differ in the various spectra can be ascribed to the differing protons of the N^4-acyl residues. The doubling of the peaks in the region of the resonance of the CH_3CO or CH_2CO groups is explained by the presence of cis-trans isomerism of the amide grouping. Thus, the doublet with an intensity of three protons in the case of the acetyl derivative and the multiplet with an intensity of two protons in the case of the propionyl derivative (2-2.14 ppm) are converted by heating to a narrow singlet and quartet, respectively (A_2 part of a system of the A_2B_3 type) (see the dashed lines in Fig. 3). Simultaneously, the weak peaks at 4.2-6 ppm unite in pairs to give two broad peaks at $\delta \sim 6$ (C_5-H) and 4.3 ppm (C_3-H).

Phenylacetic acid (IV, R = C_6H_5) and neutral compound V were isolated by the hydrolysis of N^4-propionylbenzylpenilloic acid lactam by aqueous alkali. The high intensity of the molecular ion peak with m/e 228 (see Fig. 1) in the mass spectrum of the compound obtained attests to the presence in it of a stable cyclic structure. The presence of ion peaks with m/e 172 $(M-56)^+$ and 171 $(M-57)^+$ indicates the presence of an N-propionyl group. Each of the three indicated ions loses a thioacetone molecule, which is typical for penicillin derivatives and isomers which contain a thiazolidine ring. In the process, fragments with m/e 154, 98, and 97, respectively, are formed. On the basis of the above and the results of elementary analysis, lactam structural formula V was proposed for this compound; structure V agrees with the IR spectrum, in which there are frequencies which correspond to a disubstituted amide with a cisoid structure (primary amide band at 1690 cm^{-1}, absence of a secondary amide band, ν_{NH} absorption at 3215 cm^{-1}), and a band at 1670 cm^{-1} which is associated with the vibration of the carbonyl group of a tertiary amide. It is apparent from the PMR spectrum of amide V (Fig. 3) that the restricted rotation of the N^4-amide grouping is preserved, as indicated by the splitting of the peaks from C_3-H and C_5-H as well as the CH_3CH_2CO grouping. Apropos of the signals from C_3-H and C_5-H, it should be noted that the more intense peak of the C_5-H doublet is found at weaker field that the weaker peak, while the opposite is true for the C_3-H doublet. This is precisely what should be expected if the appearance of the doublet is due to rotational isomerism of the propionyl grouping. Both protons are found in almost diametrically opposed positions relative to this grouping, and consequently, the direction of the effect of the magnetic anisotropy of this grouping during its rotation by 180° will be opposite for C_3-H and C_5-H.

The isolation of V from the hydrolyzate unambiguously speaks in favor of structure III and contradicts structure II.

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*In the spectra of compounds which have two carbonyl groups bonded to a nitrogen or oxygen atom one usually observes splitting of the carbonyl bands into two components which correspond to the symmetrical w_s (high-frequency) and asymmetric w_{as} (low-frequency) vibrations of the carbonyl group [6], and the first band is of lower intensity [7].

Product VI, to which the ethyl N^4-propionylbenzylpenilloate structure was assigned, was isolated along with phenylacetic acid and lactam V by aqueous-alcohol alkaline hydrolysis of N^4-propionylbenzylpenilloic acid lactam. The structure of VI was confirmed by the mass-spectrometric data. The major paths for the decomposition of the molecular ion (molecular weight 392) are the same as for benzylpenilloic acid and its N^4-acyl derivative.* The IR spectral data also confirm the structure assigned to VI: the bands at 3260 and 3070 cm^{-1} correspond to ν_{NH} of an amide; those at 1660 and 1570 cm^{-1} correspond to primary and secondary amide bands; and that at 1640 cm^{-1} is the carbonyl band for a trisubstituted amide (the higher value for the amide band for the tertiary amide in the lactams obtained is apparently explained by the different effects of the environment); and 1740 and 1210 cm^{-1} are the vibrations of the carbonyl group and C−O bond in the ester.

Thus, the N^4-acylpenilloic acid lactam structure was assigned to the products of the reaction of penilloic acids with acid anhydrides on the basis of a chemical and physicochemical (IR, UV, PMR, and mass spectra) study.

EXPERIMENTAL

Preparation of N^4-Acylpenilloic Acid Lactams. The penilloic acid (500 mg) was refluxed for 2 min with 1-2 ml of the appropriate anhydride, and the solution was vacuum evaporated with heating while adding methanol or ethanol. The resulting oil was triturated with ethanol until a white powder was obtained, which was then recrystallized several times from dibutyl ether.

Aqueous Alkaline Hydrolysis of N^4-Propionylbenzylpenilloic Acid Lactam. A total of 12.5 ml of 0.5 N NaOH was added to 884.8 mg (2.5 mmole) of N^4-propionylbenzylpenilloic acid lactam dissolved in 50 ml of dioxane, and the mixture was held at room temperature for 19 h and then titrated with 0.1 N HCl in the presence of phenolphthalein. Each mole of starting lactam consumed 1.15 moles of alkali. The neutralized solution was evaporated to one-fourth of its original volume with a rotary evaporator at no higher than 25°, and the residue was acidified with 10% H_2SO_4 to pH 2 and extracted with chloroform. The chloroform solution was washed several.times with 5% $NaHCO_3$ and then with distilled water. Removal of the solvent in vacuo gave 277.2 mg of a colorless powder which was recrystallized from ethyl acetate to give crystalline amide V with mp 172.5-173.5°. Found %: C 52.88, 52.69; H 7.08, 7.05; S 13.67, 13.90; N 11.94. $C_{10}H_{16}N_2O_2S$. Calculated %: C 52.60; H 7.06; S 14.04; N 12.27. Phenylacetic acid with mp 75-76° was isolated from the sodium carbonate solution and was identified from the IR and mass spectra.

Aqueous-Alcohol Alkaline Hydrolysis of N^4-Propionylbenzylpenilloic Acid. A total of 60 ml of 0.1 N NaOH was added to a solution of 1 g (2.88 mmole) of N^4-propionylbenzylpenilloic acid in 350 ml of ethanol. The mixture was kept at room temperature for 4 h and then evaporated with a rotary evaporator at no higher than 25° to one-fifth of its original volume. The residue was acidified with 10% H_2SO_4 to pH 2 and extracted with chloroform. The chloroform solution was washed several times with 5% $NaHCO_3$ and then with water. Removal of the solvent in vacuo gave 583 mg of a white powder which, according to mass spectroscopy, consisted of V and VI. Two recrystallizations from dibutyl ether gave crystals of ethyl N^4-propionylbenzylpenilloate with mp 108.5-110°. Found %: C 60.89, 60.81; H 7.20, 7.12; S 8.26, 8.26; N 7.42, 7.46. $C_{20}H_{28}N_2O_4S$. Calculated %: C 61.19; H 7.19; S 8.17; N 7.14. Phenylacetic acid was isolated from the sodium bicarbonate solution.

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* The fragmentation of the penilloic acids and their derivatives is described in a separate communication [8].

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