A CHEMICAL AND SPECTROSCOPIC STUDY OF ALBONOURSIN

G. B. Lokshin, A. S. Khokhlov, Yu. N. Sheinker, and L. B. Senyavina

Khimiya Prirodnykh Soedinenii, Vol. 1, No. 6, pp. 395-400, 1965

Albonoursin has been obtained in the isolation and purification of the antifungal antibiotics of albofungin (producing agent Actinomyces albus var. fungatus) and nystatin (producing agent Actinomyces noursei) [1]. At the same time, in a study of the antibiotic phalamysin a compound called "component 2" was found [2]. As a result of a comparison that we have made between albonoursin and a sample of "component 2" kindly provided by R. Brown, it has been established that these substances are identical.

In 1960 a compound B-73 was described, which was obtained in an investigation of the antibiotic E-73 (producing agent Actinomyces albulus) [3]; this also proved to be identical with albonoursin.

A determination of the molecular weight and, consequently, the empirical formula of albonoursin is difficult because of its poor solubility in the majority of organic solvents. Thus, the molecular weight of albonoursin has been found to be 377 and 393 (Rast method [1]), and its composition, from the results of elementary analysis, is expressed by the formula $C_{23}H_{25}N_3O_3$. This formula does not contradict the molecular weight determined by isothermal distillation [8] in chloroform. However, this method is not very reliable when the concentration of the chloroform solution is low (because of the poor solubility of albonoursin). Brown and Kelley have found the equivalent weight of albonoursin (260) by potentiometric titration in pyridine with tetrabutylammonium hydroxide, in accordance with which they proposed for albonoursin the empirical formula $C_{15}H_{16}N_2O_2$ [4], and then $C_{16}H_{18}N_2O_2$ [5]. For a definitive answer to the question of the composition of albonoursin, we have determined the molecular weight thermoelectrically (260.3) [6] and mass-spectrometrically (M^+, m/e 256), which, in combination with the results of elementary analysis, has undoubtedly shown the correctness of the formula $C_{15}H_{16}N_2O_2$.

The UV spectrum of albonoursin in ethanol shows two absorption maxima λ_{max} 232-234 mμ, $\log \varepsilon$ 3.95 and λ_{max} 316-318 mμ, $\log \varepsilon$ 4.4. Spectra of this type, as a rule, indicate the pressence of α, β-unsaturated carbonyl groupings in the molecules (cf. [7]).

However, substituted pyrazines also have spectra of a similar nature (cf., for example [8]).

In the interpretation of the IR spectrum of albonoursin (Fig. 1) it may be assumed that its molecule contains the following structural elements:

1) > C=O group (1690 cm^{-1}), which can be assigned to an α, β-unsaturated ketone (with a long chain of conjugation) or to a cyclic amide with a small number of members (absence of a second amide band);

2) Highly conjugated C=C bonds or the carbonyl of a secondary amide (1646 cm^{-1});

3) A substituted benzene ring (inflexion ~1600 cm^{-1}, bands with ν 1500, 750, and 630 cm^{-1});

4) NH or OH groups. The latter are less likely, since there is no absorption band in the 1050-1350 cm^{-1} region.

The low solubility of albonoursin in water and in the majority of organic solvents hinders the performance of functional analysis. In studying its chemical properties, it was found that albonoursin readily undergoes bromination with the formation of a dibromide of composition $C_{15}H_{14}N_2O_2Br_2$, which we had previously given the formula $C_{23}H_{22}Br_3N_3O_3$ [9]. The consumption of bromine, determined iodometrically, the liberation of hydrogen bromide, and also a comparison of the IR and UV spectra of albonoursin and its dibromide permit us to speak with confidence of the occurrence of a substitution reaction.

Albonoursin is not oxidized by potassium permanganate and chromic anhydride in an acid medium, but readily reacts with $KMnO_4$ in alkaline solution or in pyridine with the formation of benzoic acid.

When albonoursin is heated with thionyl chloride, a product is obtained whose elementary analysis agrees well with the formula $C_{15}H_{14}Cl_2N_2O_2$. A study of the UV and IR spectra of this compound shows that its structure is apparently of the same type as that of the dibromide. Albonoursin does not undergo acylation or methylation and does not react with phenylhydrazine. The reduction of albonoursin with $LiAlH_4$ in tetrahydrofuran gave a base containing, from the results of the NMR spectrum, a monosubstituted benzene ring (δ = 7.3), an isopropyl group (δ = 1.2), two NH groups, and two CH_2 groups attached to nitrogen (δ = 8.4 and 3.1).

In alkaline solution in the cold, albonoursin couples with diazotized sulfanilic acid, and on heating it decomposes with the formation of benzaldehyde and isobutyraldehyde, which were identified in the form of the 2, 4-dinitrophenylhydrazones [9].

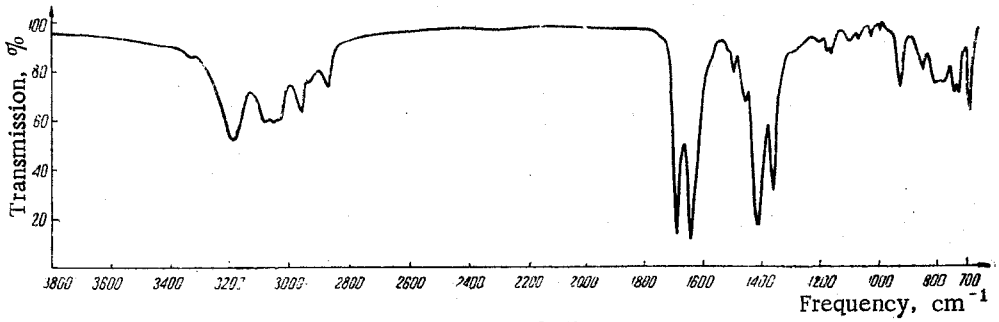


Fig. 1 IR spectrum of albonoursin.

After the acid hydrolysis of albonoursin, substance A was isolated with a molecular weight found by isothermal distillation in acetone, of 161.4; in combination with the elementary analysis, this permits it to be assigned the formula $C_9H_8O_3$. It is interesting to note that a mass-spectrometric determination of the molecular weight of substance A gave a figure of 327, which shows the dimeric state of the substance even in the vapor phase.

The UV spectrum of compound A has one absorption maximum, the position of which varies somewhat according to the nature of the solvent (Fig. 2). The IR spectrum of substance A (Fig. 3) shows the presence in it of hydroxyl groups participating in intramolecular and intermolecular hydrogen bonds (absorption bands at 3050—3200 and 3480 cm^{-1}); a >C=O group forming part of the carboxyl group of a dimer (1700 cm^{-1}); conjugated C=C bonds (1625 cm^{-1}); and a benzene ring (1500, 749, 693 cm^{-1}).

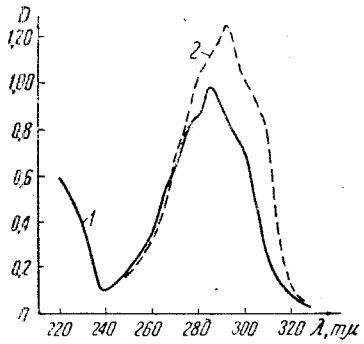


Fig. 2 UV spectrum of substance A: 1) in ethanol; 2) in chloroform.

On studying the chemical properties of substance A it was found that it is a hydroxyacid (it reacts with $NaHCO_3$ liberating CO_2, gives a coloration with $FeCl_3$, and is acetylated by $(CH_3CO_2)_2O$ in pyridine with the formation of a mono-derivative $C_{11}H_{10}O_4$). On bromination, compound A is readily converted into a monobromide $C_9H_7BrO_3$, and in alkaline solution it couples with diazotized sulfanilic acid.

By comparing the experimental results obtained for substance A and the literature data for hydroxyacids with the composition $C_9H_8O_3$ containing an aromatic nucleus we have come to the conclusion that substance A is identical with phenylpyruvic (α-hydroxycinnamic) acid.

Characteristic	Experimental results for substance A	Literature data for phenylpyruvic acid
Mp, °C	149-150	150 [10]
Mp of the monacetyl derivative, °C	160.5-161	168 [11]
Mp of the monobromo derivative, °C	98-99	102 [12]
Reaction with ferric chloride	Green	Green
Azo-coupling reaction	Positive	Not reported

The great similarity of substance A and phenylpyruvic acid is also found when their UV and IR spectra are compared (cf. [13, 14]).

We have carried out an independent synthesis of this acid by the azlactone method [15]. The results of a direct comparison of compound A and a synthetic sample showed that these substances were identical.

In addition to phenylpyruvic acid, a mixture of 2, 4-dinitrophenylhydrazones of carbonyl-containing compounds was isolated from the hydrolysis products. After separation by the method that we have described previously [9], the 2, 4-dinitrophenylhydrazones of benzaldehyde and acetone were isolated and identified by direct comparison with synthetic samples. On the basis of the quantitative ratios of the products isolated, it may be assumed that in acid hydrolysis benzaldehyde is the product of side reactions or secondary reactions.

A combination of the results obtained in the study of albonoursin (see chart) enables us to assume the presence of the following structural elements in its molecule:

1) a $C_6H_5CH{=}C{-}C$ grouping (formation of benzaldehyde on alkaline hydrolysis and of phenylpyruvic acid on acid hydrolysis);

2) a $(CH_3)_2CH-C=$ or $(CH_3)_2C=C$ grouping (formation of isobutyraldehyde on alkaline hydrolysis and of acetone on acid hydrolysis);

3) two amide groups CONH (production of more than 1 mole of ammonia on acid and alkaline hydrolysis and absence of reactions for a ketone carbonyl group although a carbonyl frequency is present in the IR spectrum, formation of a diamine on reduction with $LiAlH_4$

4) Two hydrogen atoms capable of being replaced by bromine atoms (by the action of $SOCl_2$).

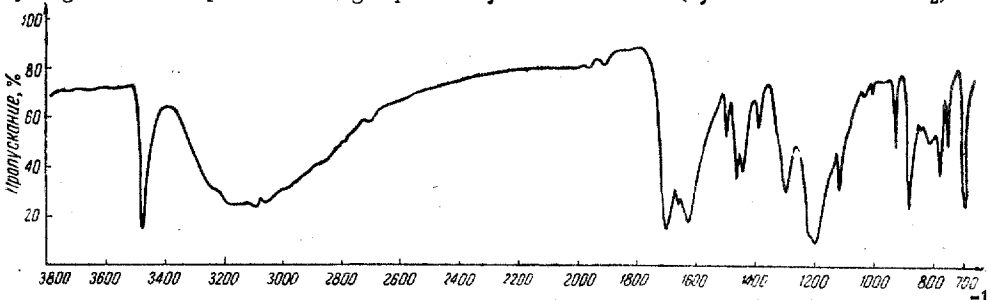
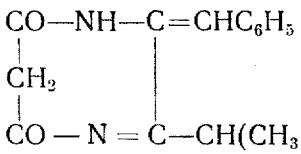
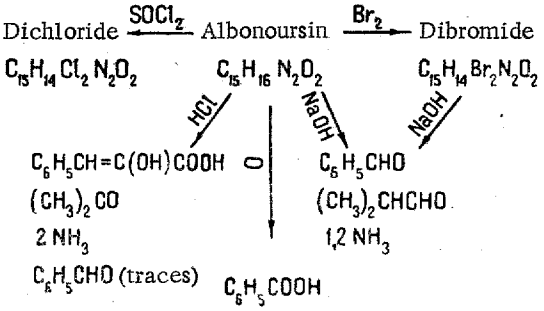


Fig. 3 IR spectrum of substance A.

But, in spite of the definite nature of a large number of the groupings contained in the molecule of albonoursin, it has not yet been possible to establish its full structural formula. All the reactions of albonoursin can be explained more or less convincingly by taking the following formula as a basis



However, the absence of a CH_2 group connected to two other CH_2 groups from the NMR spectrum of the base obtained by the reduction of albonoursin with $LiAlH_4$ is against formula (I). In addition, it does not explain some chemical properties and spectral results.



Experimental

Bromination of albonoursin. A. A mixture of 208 mg (0.82 mmole) of albonoursin, 75 ml of chloroform, and 21 ml of a 4% solution of Br_2 in chloroform was kept in the dark for 20 hr at room temperature, after which it was evaporated to dryness under vacuum. The residue was recrystallized twice from butanol. This yielded 150 mg of a white crystalline product with mp 222-223°C (decomp).

Found, %: C 44.25; H 3.65; N 6.88; Br 38.18. Calculated for $C_{15}H_{14}Br_2N_2O_2$ %: C 43.48; H 3.38; N 6.73; Br 38.65.

B. A mixture of 345 mg (1.35 mmole) of albonoursin and 75 ml of CH_3COOH was heated with stirring to 60-65°C, after which a solution of 1.4 g (8.7 mmole) of Br_2 in 10 ml of CH_3COOH was added to it dropwise over 0.5 hr.

After being kept for 4 hr at the boil, the solution was cooled; the precipitate which was deposited was filtered off, washed with water, dried over P_2O_5 and recrystallized from butanol. The yield of white crystalline product with mp 223-224°C was 84 mg.

The samples of dibromide obtained in acetic acid and in chloroform had identical IR spectra and gave no depression of the melting point in admixture with one another.

Oxidation of albonoursin. A. A mixture of 256 mg (1 mmole) of albonoursin and 10 ml of pyridine was heated with stirring to 50-55°C, and then 3.2 g (19 mmole) of finely-ground $KMnO_4$ was added to the solution in portions over 1 hr. After 4 hr standing at the same temperature, the resulting suspension was poured with cooling into 175 ml of 15% sulfuric acid, and the mixture was filtered. An ethereal extract of the filtrate, dried over Na_2SO_4, was evaporated to dryness, and the residue was sublimed. This gave a product (110 mg) with mp 119-120°C, which gave no depression of the melting point in admixture with benzoic acid and had an IR spectrum identical with it. Yield 90% (of theoretical).

B. A mixture of 495 mg (1.93 mmole) of albonoursin, 36 ml of 30% caustic soda, and 30 ml of 5% potassium permanganate was heated in a sealed tube for 48 hr at 100-105°C. After cooling, the suspension was filtered from sludge. The filtrate was acidified with 30% sulfuric acid with cooling, and was extracted with ether. The ethereal extract was washed with 50 ml of 5% $NaHCO_3$ solution and then the latter was made acid to congo red and again extracted with ether. The extract, after drying over Na_2SO_4, was evaporated to dryness, and the residue was recrystallized from water. This gave 70 mg (30%) of benzoic acid, mp 118-119°C.

Production of albonoursin dichloride. A mixture of 550 mg (2.14 mmole) of albonoursin and 35 ml of carefully redistilled $SOCl_2$ was boiled in a flask with a reflux condenser for 12 hr, after which the suspension was evaporated to dryness. This yielded 474 mg of a substance with mp 249-250°C (from butanol).

Found, %: C 55.79; H 4.52; N 8.46; Cl 21.95. Calculated for $C_{15}H_{14}Cl_2N_2O_2$ %: C 55.38; H 4.31; N 8.6; Cl 21.85.

Reduction of albonoursin with $LiAlH_4$ in tetrahydrofuran. 250 mg (0.97 mmole) of albononoursin in a paper thimble was charged into a Soxhlet extractor, and a suspension of 200 mg of $LiAlH_4$ in 17 ml of absolute tetrahydrofuran was placed in the flask. The reaction was carried out for 24 hr at the boil. The complex and excess of $LiAlH_4$ were decomposed by the method described by Micović and Mihailović [16]. The residue was filtered off, and the mother liquors were evaporated under vacuum. The residue was dissolved in chloroform, and the chloroform solution was extracted with hydrochloric acid (1 : 2). The acid extract was made alkaline with 15% caustic soda solution to pH 10 and was extracted with ether. This gave 37 mg of a brown amorphous base.

Acid hydrolysis of albonoursin. A mixture of 500 mg (1.9 mmole) of albonoursin, 34 ml of glacial acetic acid, and 17 ml of concentrated hydrochloric acid (d = 1.19) was heated in a sealed tube for 24 hr at 100-105°C. At the end of the reaction, the hydrolyzate was evaporated to dryness under vacuum in a current of CO_2 with freezing out of the volatile products. The residue was treated with 12 ml of chloroform and 12 ml of water. After separation, the aqueous layer was washed six times with chloroform (2 ml each), and the chloroform layer was washed with water. The combined chloroform extract was evaporated in a current of CO_2, and the residue was recrystallized from chloroform. This gave 200 mg of a white crystalline substance, which, after repeated recrystallization from benzene, had mp 149-150°C.

Found, %: C 66.32; H 4.9. Calculated for $C_9H_8O_3$, %: C 65.85; H 4.88. Mol. wt.: found – 161; calculated – 164.

The reaction of substance A with an alcoholic solution of ferric chloride gave an emerald-green coloration. In alkaline solution the product coupled with diazotized sulfanilic acid.

Acetylation. A mixture of 100 mg of substance A, 2 ml of $(CH_3CO)_2O$, and 1.2 ml of pyridine was left for 2 days at room temperature; after distillation under vacuum and the recrystallization of the residue from benzene and chloroform, 67 mg of a monoacyl derivative was obtained with the composition $C_{11}H_{10}O_4$, mp 160.5-161°C.

Found %: C 64.31; H 4.84. Calculated for $C_{11}H_{10}O_4$, %: C 64.08; H 4.85.

Bromination. A mixture of 198 mg (1.2 mmole) of substance A in 200 ml of chloroform and 197 mg (1.2 mmole) of Br_2 was left for 2 days at room temperature; subsequent distilling of the solvent under vacuum without heating in a current of CO_2 and two recrystallizations of the residue from isooctane gave a product with the composition $C_9H_7BrO_3$, mp 98-99°C.

After the thawing out of the acetic acid solution of the volatile products from the acid hydrolysis, a 40% solution of caustic soda was added to pH 9.5-10 and the mixture was steam-distilled. From the distillate a mixture of hydrazones was isolated, and this was separated by means of chromatography on alumina into two separate substances, benzaldehyde 2, 4-dinitrophenylhydrazone and acetone 2, 4-dinitrophenylhydrazone, which were shown to be identical with synthetic samples.

When a saturated solution of 2, 4-diphenylhydrazine in 2 N HCl was added directly to the acetic acid distillate, a yellow crystalline substance was obtained which gave a spot at the start on thin-layer chromatography in the hexane–ether (2 : 1) system.

The crude albonoursin was given to us by G. I. Kleiner (Riga Medicinal Products Factory). The analyses were carried out under the direction of M. N. Chumachenko, and the NMR spectra were taken by V. I. Sheichenko. The IR spectra of the substances in the crystalline s ate were taken on a UR-10 infrared spectrophotometer (Zeiss).

Summary

1. A direct comparison of albonoursin with "component 2" isolated from the producing agent of phalamysin (a mutant of Actinomyces noursei) has established their identity.

2. Phenylpyruvic acid and acetone have been identified as products of the acid decomposition of albonoursin.

3. The presence of the following groupings in the albonoursin molecule has been shown: $C_6H_5-CH=C-C$, $(CH_3)_2CH-C=$ or $(CH_3)_2C=C$, two amide groups, and two hydrogen atoms readily capable of being replaced by halogen atoms

(under the action of Br_2 or $SOCl_2$).

4. There is no malonamide group in albonoursin.

REFERENCES

1. G.S. Rozenfel'd et al., Antibiotiki, 8, 201, 1963.
2. R. Brown, and C. Kelley, Annual Report of Division of Laboratories and Research, N.Y. State Department of Health, Albany, 10 1957.
3. K. Rao and W. Cullen, J. Am. Chem. Soc., 82, 1127, 1960.
4. C. Kelley and R. Brown, Anual Report of Division of Laboratories and research, N. Y. State Department of Health, Albany, 75, 1962.
5. R. Brown and C. Kelley, Annual Report of Division of Laboratories and Research, N. Y. State Department of Health. Albany, 77, 1963.
6. W. Simon and C. Tomlinson. Chim., 14, 303, 1960.
7. A. Gillem and E. Stern, An Introduction to Electronic Adsorption [Russian translation], Moscow, 133, 1957.
8. G. Newbold, J. Chem. Soc., 2679, 1951.
9. A.S. Khokhlov and G.B. Lokshin, DAN SSSR, 148, 1320, 1963.
10. W. Wislicenus Ber., 20, 592, 1887.
11. W. Dieckmann, Ber., 43, 1034 1910.
12. B. Sobin and G. Bachmann, J. Am chem. Soc., 57, 2458, 1935.
13. M. Boe and M. Gex, C. r. Acad. sci, 204, 770, 1937.
14. M. Josien and M. Joussot-Dubien, J. Vizet, Bull. Soc. Chim France, 1148, 1957.
15. Collection: Organic Synthesis [Russian translation], Moscow, 2, 518, 1949.
16. V. Micovic and M. Mihailovic, J. Org. Chem., 18, 1190, 1953.

2 June 1965

Institute of the Chemistry of Natural Compounds AS USSR

All-Union Scientific Research Institute for Antibiotics