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1963

SovietRxiv

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Abstract

Full Text

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SOME CLEAVAGE PRODUCTS OF ALBONOURSIN

(Presented by Academician M. M. Shemyakin, 12 VII 1962)

In studying methods for the purification of the antifungal antibiotics albofungin, formed by *Actinomyces albus* var. *fungatus* (^{1}), and nystatin (from *Actinomyces noursei*) (^{2}), it was found that they are accompanied by one and the same crystalline substance, $C_{23}H_{25}N_3O_3$ (m.p. 257-259°), named albonoursin (^{3}). The present paper reports data on the structure of its cleavage products.

Albonoursin is insoluble in water and in solutions of sodium bicarbonate and carbonate, but is readily soluble even in one-percent alkali. On acidification of alkaline solutions shortly after dissolution, unchanged albonoursin separates, as was demonstrated by its melting point and its UV spectrum (Fig. 1). On heating alkaline solutions, a sharp bitter-almond odor appears, indicating possible cleavage of one or several aromatic aldehydes.

Fig. 1. UV spectra. a —initial albonoursin, b —albonoursin reprecipitated from alkali, v —tribromoalbonoursin.

Alkaline hydrolysis of albonoursin. 105 mg of albonoursin are dissolved in 30 ml of 30% NaOH and treated with steam for 3 hours. To the aqueous distillate is added a saturated solution of 2,4-dinitrophenylhydrazine (DNPH) in $2N$ HCl, and the mixture is left at 4°. The precipitate is filtered off, washed with water until the acid reaction disappears, and dried at 95-100°.

Study of the resulting precipitate by thin-layer chromatography on alumina (^{4}) in the system hexane—diethyl ether (2 : 1) showed that it is a mixture of at least three substances (Fig. 2), of which the starting one is unreacted DNPH, while the other two are hydrazones. The hydrazone spots differ in color: the upper one is yellow, and the lower one orange. It is possible that small amounts of other hydrazones are also present in the mixture, not detected because of their low concentration.

Separation of the 2,4-dinitrophenylhydrazones is carried out in two stages:

Fig. 2. Chromatogram of a mixture of hydrazones. a –yellow spot, b –orange spot, c –spot of unreacted DNPH

Figure 2: Fig. 2. Chromatogram of a mixture of hydrazones. a –yellow spot, b –orange spot, c –spot of unreacted DNPH

a cruder one, by extraction with 35% ethanol on heating, and a thorough one, by chromatography on alumina. 600 mg of the obtained mixture of hydrazones are treated twice, at boiling, with 75 ml of 35% ethanol, and the filtered mother liquor is left at 4°. The precipitate that falls overnight is filtered off, washed with a small amount of 35% alcohol, dried at 60–70°, dissolved in 40 ml of a mixture of hexane with diethyl ether (2 : 1), and chromatographed on a column 15-16 mm in diameter containing 50 g of Al_2O_3 (activity grade II). For development and elution of the substances, the above-mentioned solvent mixture is used.

On the column, after development, three zones are formed: the upper one–red, the middle one–orange, and the lower one–yellow. The yellow zone is eluted; the eluate is evaporated to dryness, and the precipitate is recrystallized from 35% ethyl alcohol. The product obtained (m.p. 177-179°), on rechromatography on a plate, gives only one spot with R_f = 0.6–0.65. The isolated substance, which according to elemental analysis had the composition $C_{10}H_{12}N_4O_4$, was identified as the 2,4-DNP-hydrazone of isobutyraldehyde by direct comparison with a specially synthesized specimen of the latter (5, 6), by coincidence of melting points and IR spectra (Fig. 3) and by the absence of a depression of the melting point of a mixed sample.

From the fraction sparingly soluble in 35% ethanol, by chromatography on alumina and subsequent recrystallization from 95% alcohol, a second 2,4-DNP-hydrazone with m.p. 234-235° was isolated. Its analysis and melting point are, within experimental error, close to those of the 2,4-DNP-hydrazones of *p*-tolualdehyde and benzaldehyde. For precise identification of the product obtained, the 2,4-DNP-hydrazones of *p*-tolualdehyde and benzaldehyde were synthesized. On comparison it was found that the isolated 2,4-DNP-hydrazone is completely identical with the 2,4-DNP-hydrazone of benzaldehyde in analysis, melting point, absence of depression of the melting point of a mixed sample, and IR spectra (Fig. 4).

Fig. 2. Chromatogram of a mixture of hydrazones. *a* –yellow spot, *b* –orange spot, *c* –spot of unreacted DNPH

Thus, of the 23 carbon atoms of albonoursin, the nature of 11 atoms may be considered established, as being part of the residues of isobutyric and benzoic aldehydes. The structure of the remaining part of the albonoursin molecule has not yet been clarified, although some data have already been obtained that make it possible to judge its chemical nature. Thus, albonoursin in chloroform solution reacts readily with bromine in the cold and in the dark, forming a tribromo derivative. The latter was isolated after treatment of the reaction–

Fig. 4. IR spectra. a —hydrazone of p-tolualdehyde, b —isolated hydrazone, c —hydrazone of benzaldehyde

Figure 3: Fig. 4. IR spectra. a —hydrazone of p-tolualdehyde, b —isolated hydrazone, c —hydrazone of benzaldehyde

Fig. 3. IR spectra. *a* —hydrazone of isobutyric aldehyde,
b —isolated hydrazone

mixture with an aqueous bisulfite solution, washing with water, evaporating to dryness in vacuo, and recrystallizing from butanol. The product obtained was a white crystalline substance with m.p. 221-222° (decomp.). The empirical formula according to elemental-analysis data was $C_{23}H_{22}N_3O_3Br_3$.

Found, %: C 44.25; H 3.65; N 6.88; Br 38.18
$C_{23}H_{22}N_3O_3Br_3$. Calculated, %: C 43.93; H 3.5; N 6.68; Br 38.22

The consumption of bromine, calculated on the basis of iodometric titration data in bromination in $CHCl_3$ and glacial acetic acid, proved on average to be 6.2 (in $CHCl_3$) and 5.8 (in CH_3COOH) atoms of bromine per mole of albonoursin, which makes it possible to speak of the occurrence only of a substitution reaction. The UV spectrum of the tribromide in $CHCl_3$ has the same maximum as that of the starting albonoursin.

Fig. 4. IR spectra. *a* —hydrazone of *p*-tolualdehyde, *b* —isolated hydrazone, *c* —hydrazone of benzaldehyde

Alkaline solutions of albonoursin give an azo-coupling reaction with diazotized sulfanilic acid, forming a red pigment. The tribromo derivative does not give this reaction. Alkaline hydrolysis of tribromoalbonoursin leads to the formation of the same aldehydes as are obtained from the starting substance, from which it may be concluded that bromine does not enter the aromatic nucleus responsible for the formation of benzaldehyde.

The authors express their gratitude to G. I. Kleiner (Riga Medicinal Preparations Plant) for providing crude albonoursin, to L. B. Senyavina for determining the IR spectra, and to M. N. Chumachenko, under whose supervision the microanalyses were carried out.

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of Antibiotics

Received
5 VII 1962

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Note: Figure translations are in progress. See original paper for figures.