WATER-SOLUBLE STEROL DERIVATIVES

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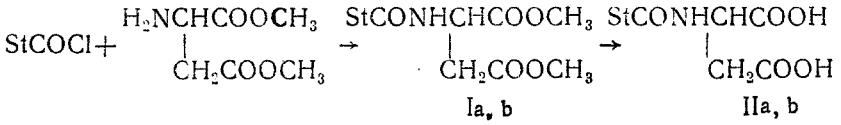
UDC 547.922:547.477

Methods have been developed for obtaining water-soluble cholesterol and sitosterol derivatives which consist in the reaction of the sterol chloroformate with dimethyl aspartate followed by saponification or in the reaction of the sterol with anhydroacetylcitric acid chloride and hydrolysis.

Water-soluble sterol derivatives are obtained by adding to the sterol hydroxyl groups bearing acidic or basic functions. The solubility in water of the monoesters of cholesterols and dicarboxylic acids that have been described in the literature is low [1]. An increase in the number of carboxy groups will permit compounds suitable for intravenous infusions to be obtained [2]. The methods that have been used for synthesizing such compounds — the reaction of glutaric acid α-isocyanate with sterols [3] and the reaction of sterols with butanetetracarboxylic dianhydride [2] — possess a number of disadvantages: the first method requires the prolonged treatment of the steroid at a high temperature and the second leads to to the formation of a mixture of isomeric esters.

In the present paper, we consider other methods of obtaining water-soluble sterol derivatives. For the addition to a sterol hydroxyl of a dibasic residue we used the reaction of the sterol chloroformate with dimethyl aspartate, followed by hydrolysis:

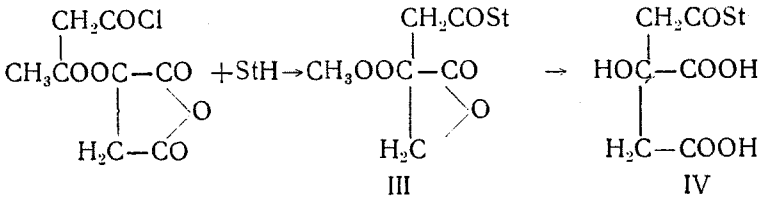


Ia, b — IIa, b

St—cholesterol (a), β-sitosterol (b).

Hydrolysis was carried out under mild conditions excluding the cleavage of the urethane group, as was confirmed by the quantitative reconversion of the acid into the ester.

Another method is the reaction of the sterol with anhydroacetylcitric acid chloride followed by the hydrolysis of the monoester obtained. The monoesters of anhydroacetylcitric acid open the anhydride ring on dissolution in water, but the sterol esters were stable and the ring was opened only on heating in the presence of hydrochloric acid. The acetyl group was split out simultaneously:



III — IV

The splitting out of the acetyl group was confirmed by the results of elementary analysis and with the aid of IR spectroscopy. The acid (IVa) obtained after hydrolysis was converted into an ester by treatment by diazomethane. The IR spectrum of the mixed esters so obtained had an absorption band with ν 3550 cm^{-1}, identical in position and in intensity with the absorption band of the hydroxy group of trimethyl citrate.

Institute of Experimental Medicine, Academy of Sciences of the USSR, Leningrad. Translated from Khimiya Prirodnykh Soedinenii, No. 5, pp. 655-657, September-October, 1980. Original article submitted May 5, 1980.

 0009-3130/80/1605-0472$07.50

Disodium salts of the cholesterol and sitosterol derivatives synthesized, particularly salts of the citric acid monoesters, possessed a considerable (up to 5%) solubility in water. When solutions were injected into animals in a chronic experiment, they readily tolerated them.

EXPERIMENTAL

Cholesterol chloroformate was obtained by the method of Kucherov and Kocheshkov [4], β-sitosterol by that of Campbell and Shepherd [5], dimethyl aspartate by that of Chambers and Carpenter [6], and anhydroacetylcitric acid by that of Easterfield and Salls [7]. IR spectra were taken in chloroform on a UR-20 instrument, and TLC was performed on Silufol plates with detection by iodine vapor. The analysis of all the compounds corresponded to the calculated figures.

Dimethyl N-Cholesteryloxycarbonylaspartate (Ia). A solution of 4 g of the hydrochloride of dimethyl aspartate in 5 ml of methanol was treated with a solution of 2.7 ml of triethylamine in 50 ml of anhydrous ether. The precipitate was separated off, and 4.2 g of cholesterol chloroformate was added to the filtrate and the mixture was stirred for 1 h. After the new precipitate had been separated off, the filtrate was evaporated to dryness and the residue was crystallized from acetone and hexane. Yield 4.4 g (81%), mp. 128-129°C, $\nu_{max}^{CHCl_3}$, cm^{-1}; 1715, 1750 (CO), 3400 (NH).

N-Cholesteryloxycarbonylaspartic Acid (IIa). A solution of 1.5 g of (Ia) in 20 ml of acetone was treated with 9 ml of 1 N caustic potash solution and the mixture was stirred for 30-60 min, the completeness of hydrolysis being followed with the aid of TLC (benzene–ethyl acetate (2:1); Rf of (Ia) 0.56). The precipitate was separated off and was dissolved in 15 ml of water. The solution was acidified with hydrochloric acid and extracted with ether. Yield 1.2 g (86%), mp 170°C (from ethanol).

A solution of 0.2 g of the acid so obtained in 10 ml of ether was treated with an excess of diazomethane. The initial ester was obtained, being identified by its melting point, IR spectrum, and TLC.

Disodium N-Cholesterylcarbonylaspartate. A solution of 1.1 g of (IIa) in methanol was added to a solution of 5 ml of 1 N sodium methanolate in methanol, and the precipitate was separated off. Yield 0.9 g (75%).

Dimethyl sitosteryloxycarbonylaspartate (Ib), mp 122°C (from hexane) and sitosteryloxycarbonlyaspartic acid (IIb), mp 173°C (from methanol) and its disodium salt were obtained similarly.

Anhydroacetylcitric Acid Chloride. A mixture of 6.3 g of anhydroacetylcitric acid and 9 ml of thionyl chloride was boiled for 3 h, and then the solvent was distilled off and the residue was dissolved in the minimum amount of anhydrous ether. Yield 4.3 g (62%), mp. 89-92°C [8].

Cholesteryl Ester of Anhydroacetylcitric Acid (IIIa). A solution of 2.8 g of anhydroacetylcitric acid chloride and 3.8 g of cholesterol in 30 ml of benzene was boiled for 10 h, and then the solvent was distilled off and the residue was recrystallized from hexane. Yield 4.0 g (68%), mp 177-178°C, $\nu_{max}^{CHCl_3}$, cm^{-1}: 1730, 1748 (CO), 1796, 1870 (anhydride).

Disodium Salt of the 1'-Cholesteryl Ester of Citric Acid (IV). A mixture of 2 g of (IIIa), 10 ml of 20% hydrochloric acid, and 30 ml of dioxane was boiled for 3 h and was then evaporated to dryness in vacuum, and the residue was dissolved in a mixture of water and ether. The residue obtained after the distillation of the ether was dissolved in 20 ml of anhydrous ethanol and 8 ml of a 1 N solution of sodium ethanolate in ethanol was added. The precipitate was separated off and washed with acetone. Yield 1.3 g (60%).

The sitosteryl ester of anhydroacetylcitric acid (IIIb) yield 40%, mp 168-172°C (from a mixture of benzene and hexane) and the disodium salt of the 1'-β-sitosteryl ester of citric acid, yield 50%, were obtained similarly.

SUMMARY

Methods have been developed for the synthesis of water-soluble derivatives of sterols, and the corresponding derivatives of cholesterol and sitosterol have been obtained.

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PREPARATION OF TRITIUM-LABELED BIOGENIC AMINES AND THEIR ANALOGS

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UDC 546.110.23:547.565.435

The possibility has been shown of introducing a tritium label into biogenic amines and their analogs in nonaqueous systems. Labeled compounds possessing a specific activity sufficient for performing many biological investigations have been obtained in good yields.

Investigations of recent years have shown the exceptionally important role of biogenic amines in the performance of adaptive reactions of the organism. At the present time the biosynthetic pathways of these biologically active compounds have been largely elucidated and determined with accuracy, and the main enzyme systems participating in the multistage processes have also been studied. For subsequent biochemical, physiological, and chemical investigation a continuous improvement and the introduction of modern methods of determining both the biogenic amines themselves and their transformation products in the organism are necessary. One of such methods is the use of radioactive preparations [1, 2].

In the present paper we consider the introduction of a tritium label into dopamine, norepinephrine, phenylephrine, β-phenylethylamine, tyramine, and metanephrine by the method of heterogeneous catalytic isotope exchange in solution with gaseous tritium.

Since, when this type of reaction is carried out in aqueous media, intensive isotope exchange takes place between the gaseous tritium and the solvent, and also in view of the fact that many biogenic amines are labile in aqueous solutions, we used methanol as the solvent. We first studied the stability of the biogenic amines and the distribution of the isotopic label in the molecules of these compounds under the conditions that we proposed to use for the catalytic reactions with gaseous tritium. In order to determine the optimum reaction time of isotope exchange we studied the kinetics of the inclusion of 0.1% tritium into the tyramine molecule (Fig. 1). We simultaneously determined the tyramine content by spectral methods (UV). As can be seen from Fig. 1, after about 3 h the rate of inclusion of the label decreases substantially and the concentration of tritium has fallen by only 5%, and therefore it was decided to limit the reaction time to three hours.

In studying the stability of the structures investigated, we treated norepinephrine and metanephrine with gaseous deuterium (pressure 250 mm Hg) in methanol for 3 h and, without additional purification, analyzed the compounds obtained by the method of PMR, UV, and mass spectroscopy.

The mass spectra of the deuterated and initial norepinephrine and metanephrine are shown in Fig. 2. As can be seen from a comparison of spectra a and b and of spectra c and d, the molecular peaks and the main peaks of the fragments coincide and, therefore, the treatment of norepinephrine and metanephrine under the selected conditions is not accompanied by the decomposition of the substance.

Institute of Molecular Genetics, Academy of Sciences of the USSR, Moscow. Translated from Khimiya Prirodnykh Soedinenii, No. 5, pp. 657-663, September-October, 1980. Original article submitted April 7, 1980.

 0009-3130/80/1605-0474$07.50