methylated polyols corresponding to the methyl glycosides given above. As a result of the hydrolysis of the methyl ethers of the fatsiosides with reduction and subsequent acetylation, 1,5-di-O-acetyl-2,3,4-tri-O-methylarabitol (6), 1,5-di-O-acetyl-2,3,4-tri-O-methylrhamnitol (7) and 1,4,5-tri-O-acetyl-2,3,6-tri-O-methylsorbitol (8) were identified for fatsioside D, and 1,5-di-O-acetyl-2,3,4,6-tetra-O-methylsorbitol (9) and 1,2,5-tri-O-acetyl-3,4-di-O-methylarabitol (10), and also the polyols (7) and (8), for fatsiosides E and F [4].

On the basis of the facts given above, the polar glycosides from the leaves of *Fatsia japonica* can be assigned the following structures: fatsioside D — hederagenin 3-O-L-Ara$_p$ 28-O-L-Rha$_p$-(1 → 4)-D-Glc$_p$-(1 → 4)-D-Glc$_p$; fatsioside E — oleanolic acid 3-O-D-Glc$_p$-(1 → 2)-L-Ara$_p$ 28-O-L-Rha$_p$-(1 → 4)-D-Glc$_p$-(1 → 4)-D-Glc$_p$; and fatsioside F — hederagenin 3-O-D-Glc$_p$-(1 → 2)-L-Ara$_p$ 28-O-L-Rha$_p$-(1 → 4)-D-Glc$_p$-(1 → 4)-D-Glc$_p$.

Glycosides of similar structure have been isolated by Japanese scientists from the fruit of *Fatsia japonica* [5].

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# CASEINIC ACID DERIVATIVES OF CYTISINE AND SALSOLIDINE


V. A. Degtyarev, V. K. Burichenko, and Yu. D. Sadykov



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It is possible to prolong the action of drugs by adding physiologically active substances to proteins [1]. Berberine caseinate has been obtained previously [2] with the aim of lowering toxicity and creating a prolonging effect. The basis for such reactions was that casein can be considered as a polybasic acid because of the presence of a large number of carboxy groups belonging to aspartic and glutamic acid residues in the molecule. For the synthesis we took casein obtained in the form of caseinic acid [3]. We condensed the alkaloids cytisine and salsolidine with the caseinic acid. The amine groups of the caseinic acid were blocked by benzyloxycarbonyl groups [4], as was shown by the absence of amine nitrogen determined by the Van Slyke method [5].

The benzyloxycarbonyl protection was subsequently eliminated with a 40% solution of HBr in glacial acetic acid. The carboxy groups of the protein were converted with ethyl chloroformate into reactive mixed anhydrides which were then used in the condensation reaction with the alkaloids. The reaction took place by the following scheme:

$$m\,C_6H_5CH_2OCOCl + (NH_2)_m\text{—Protein mol.}\text{—}(COOH)_n \xrightarrow[\text{pH 8,5}]{0^\circ} (CbO\text{—}NH)_m\text{—}$$

$$\text{—Protein mol.}\text{—}(COOH)_n \quad (I)$$

$$(I) + NH[\text{Alkaloid}] \xrightarrow[\text{pH 7,5};(C_2H_5)_3N;\,ClOCOC_2H_5]{0^\circ} (CbO)\text{—}NH)_m\text{—Protein mol.}\text{—}$$

$$(CO\text{—}N[\text{Alkaloid}])_n \quad (II)$$

$$(II) + \xrightarrow[CH_3COOH]{HBr} (HBrNH_2)_m\text{—Protein mol.}\text{—}(CO\text{—}N[\text{Alkaloid}]) \quad (III)$$

The purity of the products was checked by thin-layer chromatography on silica gel fixed with gypsum in the following systems: 1) butanol—water—acetic acid (4:1:1), and 2) benzene—ethanol (85:15). The revealing agent for the alkaloids was the Dragendorff reagent. The


V. I. Nikitin Institute of Chemistry of the Academy of Sciences of the TadzhSSR, Dushanbe.


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condensation products gave a positive reaction for alkaloids with tungstosilicic acid. A spectrophotometric study of the hydrolysates for their cytosine and salsolidine contents showed that the amount of cytosine in the caseinylcytosine was 12% and the amount of salsolidine in the caseinylsalsolidine 7%.

Benzyloxycarbonylcasein (I). A solution of 2 g of caseinic acid in 10 ml of 2 N NaOH at pH 8.5 was cooled to 0°C and, with stirring, 8 g of benzyloxycarbonyl chloride was added dropwise over 30 min, and then the reaction mixture was treated twice with diethyl ether and was acidified with 10 N HCl. The resulting precipitate was washed with $H_2O$, $C_2H_5OH$, and absolute ether and was dried over $H_2SO_4$. The yield of product was 3 g. A Van Slyke determination of amine nitrogen [5] showed that it was absent.

Benzyloxycarbonylcaseinylcytosine (II). A solution of 0.4 g of benzyloxycarbonylcasein and 1.5 ml of triethylamine in 10 ml of chloroform was cooled to −5°C and 2 ml of ethyl chloroformate was added, followed after 15 min by the dropwise addition of a chloroform solution (15 ml) containing 0.1 g of cytosine and 1.5 ml of triethylamine. The solution was stirred for 30 min. The reaction mixture was washed successively with $H_2O$, 1 N HCl, 0.5 N $NaHCO_3$, and $H_2O$ and was dried over $Na_2SO_4$, and the solvent was driven off in a rotary evaporator. This gave benzyloxycarbonylcaseinylcytisine in the form of an oily product with a yield of 0.2 g. The product was soluble in methanol, ethanol, ethyl acetate, and chloroform, and was insoluble in ether and water. Benzyloxycarbonylcaseinylsalsolidine was obtained similarly with a yield of 0.25 g.

Caseinylcytosine Hydrobromide (III). To eliminate the benzyloxycarbonyl protection, 0.3 g of a 40% solution of HBr in glacial acetic acid was added to the benzyloxycarbonylcaseinylcytisine at room temperature and the mixture was left for 1.5 h, after which caseinylcytosine hydrobromide was precipitated with anhydrous diethyl ether and it was washed with ether until the HBr had been completely eliminated. The product was recrystallized from ethanol–diethyl ether (1:2). Yield 0.08 g, mp 139°C (decomp.). Chromatographic analysis of the hydrolysis product by TLC (silica gel fixed with gypsum, system 1) showed the presence of the alkaloid cytosine.

Caseinylsalsidine hydrobromide was obtained similarly, its yield amounting to 0.09 g, mp 158°C (decomp.).

Pharmacological investigations of the compounds obtained showed that their toxicity was from three to six times less than that of the initial alkaloids cytosine and salsolidine and the prolonging action was increased twofold for the caseinylcytosine hydrobromide and fourfold for the caseinylsalsolidine hydrobromide.

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