Amino Acid Studies

Part IV.* Structure and Synthesis of the Plant Amino Acid Willardiine [3-(1-Uracyl)-L-alanine]

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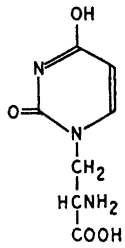
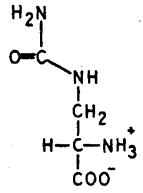
In 1959, Gmelin [1] reported the isolation of several non-protein amino acids from *Acacia Willardiana* Rose (*Mimosaceae*). One of these, named *willardiine*, was formulated as 3-(1-uracyl)L-alanine (I) on basis of its elemental composition, spectroscopic properties and stability towards strong acid. The formal similarity of (I) to L-albizziine (II) [2,3], present in the same seed material, was adduced in support of the proposed structure, particularly its steric configuration [1]. In order to provide an unambiguous proof of the structure of willardiine, the following synthetic work was undertaken.



I



II

The elegant procedure developed by Shaw *et al.*[4] for the synthesis of uracil, 1-methyl-, 1-ethyl- and 1-phenyl-uracil was adopted as the most promising synthetic approach. β-Ethoxyacryloyl chloride [5] was converted into β-ethoxyacryloyl isocyanate [4] (III) and the latter, in turn, combined with the methyl ester of L-2-(*p*-toluenesulphonamido)-3-aminopropionic acid (IV), prepared by Fischer esterification of the acid, which is readily accessible from L-asparagine [6,7].

On alkali treatment, the intermediately formed, linear urea (V) was transformed, without isolation, into L-*2-(p-toluenesulphonamido)-3-(1-uracyl)-propionic acid* (VI), a beautifully crystalline compound, which proved to be identical with a specimen of *N*-(p-*toluenesulphonyl*)-*willardiine*, produced by tosylation of the natural amino acid. Hence, willardiine possesses the structure (VII).*

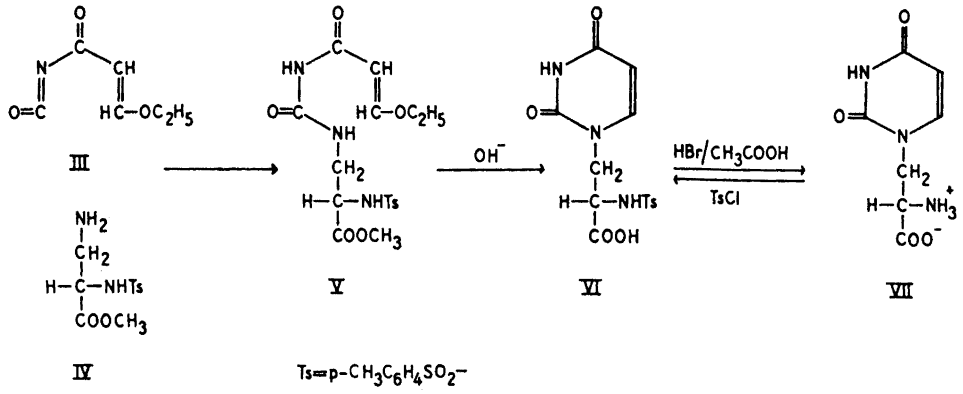
Attempts to remove the protecting group from (VI) to furnish L-willardiine were only partly successful. Detosylation experiments with sodium in liquid ammonia resulted in destruction of the uracil ring and production of at least five ninhydrin positive components, one of which was presumably diaminopropionic acid. Treatment of (VI) with anhydrous hydrogen bromide in glacial acetic acid [8] at 70° for 3 h resulted in complete removal of the protecting group. However, the reaction under these conditions seemed to be accompanied by complete racemization. Gmelin[1] proved willardiine to be stable towards hot 48 % hydrogen bromide, yet without controlling the rotation subsequent to the acid treatment. It appears likely that racemization occurred also under these conditions.

Hence, N-tosyl-L-willardiine was subjected to the same conditions as above but for only one hour. From the reaction mixture, a low yield of willardiine could be isolated, indistinguishable from the natural product with regard to rotation and infra-red spectra. In addition, some starting material was recovered as well as partly racemized fractions of willardiine. Again, it appears likely that the detosylation was accompanied by a certain decomposition, because small amounts of foreign ninhydrin positive substances were observable upon paper chromatography of the mother liquors.

Experimental. Microanalyses were performed by Mr. G. Cornali. Rotations were measured in a 1 dm tube. Infra-red spectra were

* Part III: *Acta Chem. Scand.* **14** (1960) 961.

* *Added in proof:* After this communication was submitted to publication, Shaw and Dewar [9] independently reported a synthesis of DL-willardiine. According to a private communication from Dr. G. Shaw, the racemic modification has now been resolved to give (−)-willardiine, identical with a specimen of natural origin. Clearly, this synthetic approach provides no information as to the absolute configuration of natural willardiine.



determined in potassium bromide pellets on a Perkin-Elmer "Infracord"-instrument. Melting points were determined in capillary tubes in an Anschütz-Hershberg apparatus equipped with fully immersed thermometers. The standard rate of heating was 2° per min.

L-2-(p-*Toluenesulphonamido*)-3-(*1-uracyl*)-*propionic acid* (VI). (*a*) L-2-(*p*-Toluenesulphonamido)-3-aminopropionic acid (5 mequiv.) was dissolved in anhydrous methanol, and dry HCl was introduced until the acid had dissolved. After 16 h at room temperature, the solution was evaporated to dryness and the oily residue was dried over P_2O_5 at 0.1 mm Hg for 24 h. The ester was liberated on addition of triethylamine (20 mequiv.) in anhydrous benzene (20 ml), and triethylammonium chloride filtered off. β-Ethoxyacryloyl chloride [5] (5.3 mequiv.) was shaken with dry AgOCN (7.6 mequiv.) and anhydrous benzene (20 ml) for 3 h at room temperature, and the filtered solution was introduced directly into the above ester solution. A slight evolution of heat was noticed. The solution was shaken for 4 h at room temperature, gradually depositing a slight precipitate of crystalline material. The entire mixture was taken to dryness and boiled up with NaOH (15 ml, 1 N), causing the major part of the oil to dissolve. After 15 h at 70°, subsequent cooling and ether extraction, HCl was added to bring the pH-value to 1 – 2. Crystalline L-2-(*p*-toluenesulphonamido)-3-(1-uracyl)-propionic acid thereby separated in a yield of 1.02 g (58 %). Two recrystallizations from water afforded an analytically pure specimen m.p. > 250° (decomp.), $[\alpha]_D^{25}$ −67.1° (*c* 1.0, 0.1 N NaOH), λ_{max} 264 mμ, ε 7 000; λ_{max} 227 mμ, ε 15 900; λ_{max} 207 mμ, ε 15 400; (solvent: 0.08 N NH_3). (Found: C 47.45; H 4.41; N 11.77. Calc. for $C_{14}H_{15}N_3O_6S$: C 47.58; H 4.28; N 11.90).

(*b*) Willardiine of natural origin (0.24 mequiv.) was dissolved in NaOH (6.9 ml, 0.1 N) and shaken with a solution of *p*-toluenesulphonyl chloride (0.32 mequiv.) in ether for 3 h. The ether phase was discarded and the aqueous phase was adjusted to pH 1 – 2 with HCl, when L-2-(*p*-toluenesulphonamido)-3-(1-uracyl)-propionic acid (28.8 mg) separated. Retosylation of the mother liquor afforded an additional crop (18.7 mg), thereby increasing the total yield to 57 %. Recrystallization from water afforded a pure specimen, $[\alpha]_D^{26}$ −66.0° (*c* 1.0, 0.1 N NaOH) (Found: C 47.38; H 4.43 N 11.78). The infra-red spectrum was indistinguishable from that of the specimen prepared as described under (*a*).

3-(1-Uracyl)-L-alanine (willardiine) (VII). L-2-(*p*-Toluenesulphanomido)-3-(1-uracyl)-propionic acid (1 mequiv.) was dissolved in glacial acetic acid (12 ml) saturated with HBr. After 1 h at 70°, anhydrous ether was added to the cooled solution and a spongy precipitate was obtained. After decantation and thorough washing with dry ether, the precipitate was dissolved in a small volume of water; the pH-value was adjusted to 6 with NH_3 and ethanol added, causing a light brown solid (33 mg) to separate. Reprecipitation from water-ethanol, followed by recrystallization from water, afforded 3.9 mg of a nearly colourless product, $[\alpha]_D^{26}$ −11° (*c* 0.25, 1 N HCl), comparable to the value $[\alpha]_D^{22}$ −12.1° (*c* 1.16, 1 N HCl) reported

for the natural product [1]. The two specimens gave identical R_F-values and infra-red spectra. The mother liquors from the purification gave crystalline fractions presumably representing partly racemized willardiine as estimated from infrared data.* Unchanged, crystalline L-2-(*p*-toluenesulphonamido)-3-(1-uracyl)-propionic acid (120 mg) separated from the original reaction mixture.

The authors are indebted to Dr. R. Gmelin for a specimen of natural willardiine. The present work is part of investigations supported by *Kai Hansen's Fond.*

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* *Added in proof:* On comparison of the infra-red spectra with one of authentic, synthetic DL-willardiine, kindly furnished by Dr. G. Shaw, these fractions in fact appeared to be racemic willardiine. The infra-red spectrum of the latter deviated notably from that of the L-isomeride in the solid state.

Studies on the Occurrence of Cholesterol in Water-Containing Liquid-Crystalline Form

I. The Minimum Fatty Acid Anion Concentrations Able to Transform Cholesterol Crystals into a Water-Containing Mesomorphous Form

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Information about the different forms in which cholesterol separates from aqueous solutions and in which cholesterol can occur in aqueous systems is of both physical-chemical and biological interest.

It has long been known that certain derivatives of cholesterol undergo a transformation into the liquid-crystalline state on heating (thermotropic mesomorphism). Cholesterol is also known to be transformed into the liquid-crystalline state by the action of solvents (lyotropic mesomorphism). Thus, for example, Steiger stated that cholesterol is transformed into a myelinic mesomorphous phase by water containing fatty acids and alkali [1]. Dervichian reported the formation of myelinic figures in the presence of water by mixtures of cholesterol with a number of biologically active long-chain substances, such as lysolecithin, alkylcholine chlorides, fatty acid salts, alkyl sulphates and alkyl phosphates [2]. Lawrence recently reported a series of observations on the transformation of solid crystalline cholesterol into a water-containing mesomorphous phase by the action of relatively concentrated association colloid solutions [3]. The liquid-crystalline substance that is formed in a 20 % sodium dodecyl sulphate solution containing an equimolar amount of cholesterol is stable on heating up to 195°C, where it separates into two isotropic liquids. When a solution of an association colloid such as sodium dodecyl sulphate flows over a cholesterol crystal, a gelatinous membrane composed of doubly-refracting matter is seen under the microscope to be formed immediately when the two phases come into contact. The membrane gradually increases in thickness and the solid crystal decreases in size, while at the same