Stereospecific Synthesis of *trans*-3-Methyl-2-hexenoic Acid

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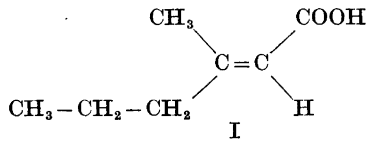
It has long been observed by psychiatrists, that there is a characteristic odour in the wards where schizophrenic patients are treated. The compound regarded to be responsible for this odour has recently been isolated from the sweat of schizophrenics and identified as *trans*-3-methyl-2-hexenoic acid (I) by Smith *et al.*[1]



I

The present investigation concerns the synthesis of this acid, a sample of which was required during a survey of schizophrenic patients being carried out by Holmstedt *et al.* at the Department of Toxicology, The Swedish Medical Research Council, Karolinska Institutet, Stockholm.

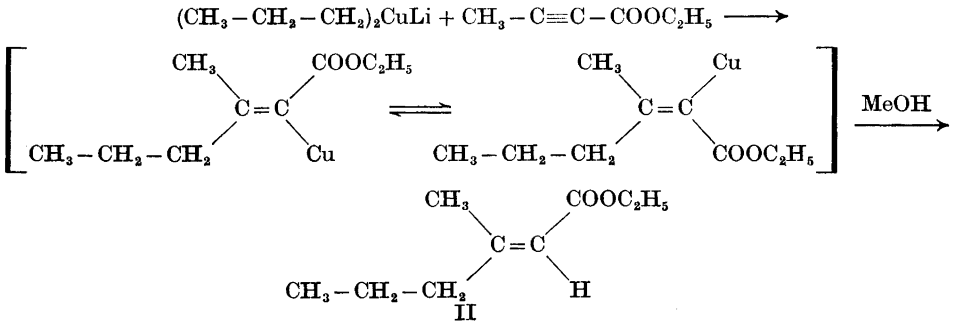
Smith *et al.* prepared *trans*-3-methyl-2-hexenoic acid by hydrolysis of ethyl *trans*-3-methyl-2-hexenoate obtained by dehydration of ethyl 3-hydroxy-3-methylhexanoate.[2] Obviously it is possible to obtain isomers in the elimination step and this has now been found to be the case. Attempts to isolate the *trans*-α,β-unsaturated isomer by preparative gas chromatography failed, giving only the *cis* isomer in the pure state.

A stereospecific synthesis of trisubstituted olefins, that has recently been described by Corey *et al.*[3] was successfully employed in the synthesis of *trans*-3-methyl-2-hexenoic acid. A propylcopper-lithium complex was added to ethyl tetrolate to give an ethylenic copper compound. Almost exclusively *cis* addition occurred when the reaction was carried out in tetrahydrofuran at −75°C. At higher temperatures equilibration becomes important and the stereospecificity is lost.[3] After methanolysis of the copper compound ethyl *trans*-3-methyl-2-hexenoate (II) was obtained.

The *trans* ester (II) was hydrolyzed in acidic medium to the acid (I). It was found that alkaline hydrolysis caused partial migration of the α,β-double bond.

The IR, UV, NMR and mass spectra of (I) were in agreement with the postulated structure.

Ethyl trans-*3-methyl-2-hexenoate.* Propyllithium in tetrahydrofuran (1.12 M) was prepared from propyl chloride at −50 to −55° essentially as described by Gilman *et al.*[4] 110 ml of cooled 1.12 M propyllithium solution was rapidly pipetted under dry oxygen-free nitrogen to a stirred suspension of 11.8 g (62 mmol) anhydrous cuprous iodide in 30 ml of purified [5] THF cooled to 0°. The mixture immediately turned blue-black with dissolution of the copper salt. The solution was cooled to −75°C and maintained at that temperature during the reaction. A pre-cooled solution of 7.0 g (62 mmol) ethyl tetrolate (b.p. 163°/760 mm) in 10 ml of dry THF was added. The reaction mixture was stirred for 3 h, then 10 ml of cold methanol was added and the mixture allowed to reach room temperature. The solution was neutralized with 2 M hydrochloric acid and the solvent evaporated *in vacuo*. 50 ml of water was added to the brown residual oil



II

and extracted with ether (3 × 100 ml). The combined ether extracts were washed with saturated sodium chloride solution, dried over sodium sulphate and the solvent evaporated under reduced pressure. The yield was 8.2 g of a brown liquid, which was purified by chromatography on neutral alumina (aluminium oxide Woelm activity grade I 80 g, 1.6 cm diam.). The ester was eluted with ether. The eluent was evaporated *in vacuo* to give 7.5 g of a slightly brown liquid. Distillation under reduced pressure afforded 6.8 g of the desired ester in 82 % purity. (GLC: 15 % Carbowax 1500 on Celite 545 60–100 mesh). B.p. 71–73°/11 mm. The ester was used in the subsequent step without further purification.

trans-*3-Methyl-2-hexenoic acid.* 1.60 g of the impure ester was dissolved in a mixture of 20 ml 1 M sulphuric acid and 25 ml of acetic acid and heated to reflux for 5 h. The solvent was evaporated *in vacuo* to approx. 5 ml, made alkaline with 2 M sodium hydroxide and extracted with ether (2 × 20 ml). The aqueous layer was acidified with 2 M hydrochloric acid and extracted with ether (3 × 50 ml). The combined ether extracts were washed with saturated sodium chloride solution, dried over sodium sulphate and the solvent evaporated *in vacuo* affording 0.67 g of the acid which crystallized on cooling. Recrystallization from ethanol-water gave 0.35 g of the desired acid as white crystals. M.p. 37–38°. The purity was judged to be better than 99 % by GLC, (10 % diethylene glycol adipate and 2 % phosphoric acid 85 % on acid-washed Chromosorb W 60–80 mesh). (Found: C 66.05; H 9.41; O 24.62. Calc. for $C_7H_{12}O_2$: C 65.60; H 9.44; O 24.96.)

Acknowledgements. I wish to thank Drs. Bernt Carnmalm, Nils E. Stjernström and Brian Pring for valuable discussions. I also wish to express my gratitude to professor Bo Holmstedt for recording the mass spectrum and Ing. Hans Thorin for performing the preparative gas chromatography.

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Received February 25, 1971.

Influence of Hydrophobic Interactions and Hydration on the Radiosensitivity of a Protein

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The radiosensitivities of organisms as well as of some macromolecules *in vitro* have been shown to be strongly influenced by the amount of hydration water present.[1–6] Generally, such systems are less sensitive in a moderately hydrated than in a desiccated form. The mechanism for this protection has been a matter of speculation only.

Another class of compounds, which has been discussed in connection with the radiosensitivities of biological macromolecules, are those which have low-lying electronic excited states. It has been suggested that such groups if present within or in the vicinity of a macromolecule might protect this by acting as "sinks" for excitation energy.[4,7–9] Such protection has been demonstrated with proteins exposed to ultraviolet light.[10,11]

In this work, the influence of hydration water and other adsorbed compounds on the radiosensitivity of bovine serum albumin has been studied in order to throw some light on these problems. Radiation injury has been assayed as the loss of solubility.

Materials and methods. Bovine serum albumin (BSA) was a crystalline preparation from Armour and Co. Results obtained with this material as such or after dissolution in distilled water and lyophilization did not deviate appreciably. DNS-BSA, the conjugate with 1-dimethylaminonaphthalene-5-sulfonate, was prepared as described in an earlier paper.[12] It had a molar ratio dye/protein of 2.7–3.0. The solid form was obtained by lyophilization.

BSA has five strong binding sites for 1-anilino-8-naphthalenesulfonate (ANS). Solid ANS-BSA was obtained by lyophilization of a concentrated solution in distilled water of the components in this molar ratio. BSA with

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