The Genometrical Structure of 3-β-Indolylacrylic Acid

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NMR-spectroscopy has proved to be a convenient tool in many fields of organic chemistry. One example is the determination of geometrical structures. *Trans*-compounds have larger spin coupling constants than *cis*-compounds; mean values of 17 – 18 cycles/sec for *trans*- and of 10 – 11 cycles/sec for *cis*-compounds are given.[1]

In connection with studies on plant growth regulators it was of interest to investigate the geometrical structure of 3-β-indolylacrylic acid.[2] This acid was obtained by condensation of 3-formylindole with malonic acid.[3] Condensation with malonic acid usually give *trans*-isomers,[4] but in the present case no geometrical structure had been assigned to the product.

The NMR-spectrum of 3-β-indolylacrylic acid was recorded and compared with several known *cis*- and *trans*-isomers of α,β-unsaturated acids. The results are collected in Table 1. Both aliphatic and aromatic acids were investigated. Besides the spin coupling constants, aromatic acids show another difference between *cis*- and *trans*-isomers. The β-hydrogens of *trans*-acids have higher δ-values than the aromatic hydrogens, while the *cis*-acids have lower δ-values.

Both these features are in accordance with a *trans* configuration for the known 3-β-indolylacrylic acid. The latter has a coupling constant of 17.5 cycles/sec and the β-hydrogen doublet has a higher δ-value than the aromatic hydrogens.

Table 1.

Acid	Spin coupling constants cycles/sec	
	cis-	*trans*-
$CH_3-CH=CH-COOH$	12	16.5
$C_2H_5-CH=CH-COOH$	12	—
$n\text{-}C_3H_7-CH=CH-COOH$	12	17
$iso\text{-}C_3H_7-CH=CH-COOH$	12	—
$BrCH=CH-COOH$	8.5	14
$ClCH=CH-COOH$	8.5	—
$C_6H_5-CH=CH-COOH$	13	17
$(4)ClC_6H_4-CH=CH-COOH$	13	17
$(3,4)Cl_2C_6H_3\ CH=CH-COOH$	13	—
$(3,5)Cl_2C_6H_3\ CH=CH-COOH$	13	17
$C_8H_7N-CH=CH-COOH$	—	17

Experimental. The NMR-spectra were recorded on a Varian Associates Model A 60 spectrometer. *3-β-Indolylacrylic acid.* Commercial sample, Sigma Chemical Company, m.p. 192 – 194°C.

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1. Pople, J. A., Schneider, W. G. and Bernstein, H. J. *High-resolution Nuclear Magnetic Resonance*, Mc Graw Hill, New York 1959, p. 238.
2. Åberg, B. *Kgl. Lantbruks-Högskol. Ann.* **25** (1959) 363.
3. Shaw, K., McMillan, A., Gudmundson, A. and Armstrong, M. *J. Org. Chem.* **23** (1958) 1171.
4. Johnson, J. *Org. Reactions* **1** (1942) 210.

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The Effect of Sodium Salicylate on Hexosamine Synthesis in Eviscerated Mouse Fetuses

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Several anti-inflammatory drugs have been found to depress the synthesis of acid mucopolysaccharides in various tissues [1,2] and in addition, to produce cleft-palate, as well as other skeletal and vascular malformations in mouse em-

bryos.[3-5] The most active of these drugs in producing both depression of polysaccharide synthesis as well as skeletal and vessel anomalies is sodium salicylate;[6] therefore the effect of this drug on several enzyme systems involved in mucopolysaccharide synthesis has been investigated. A previous study, utilizing soluble enzymes from bovine heart valves, indicated salicylate to have an inhibitory effect on the enzyme L-glutamine-D-fructose-6-phosphate amino transferase which catalyzes the synthesis of glucosamine-6-phosphate (a precursor of the galactosamine moiety of chondroitin sulfate), while the synthesis of phosphoadenosine phosphosulfate (PAPS), which eventually transfers sulfate to the galactosamine moiety of a mucopolysaccharide precursor, was not affected.[7]

In order to obtain more information regarding the possible relationship between the production of embryo malformations and inhibition of mucopolysaccharide synthesis, studies on the inhibition of hexosamine synthesis were extended to mouse fetal tissue.

Experimental. Mouse fetuses from untreated mothers of the CBA strain were taken on the 18th day of gestation and the brain and viscera removed by suction. The fetuses, approximately 20 mm in length, were cut into small pieces and dropped into liquid nitrogen, after which they were ground in a mortar at liquid nitrogen temperature. The resulting fine powder was stored in the freezer for one day before preparing the enzyme system as follows:

Experiment 1. The powder from 38 fetuses was suspended in 125 ml of an ice cold solution consisting of 0.15 M KCl-0.05 M L-glutamine-0.001 M versene-1 mg/ml glutathione, pH 7.7, and extraction carried out at 4° for 75 min with constant stirring. After centrifugation at 7000 rpm, the supernatant was taken to 75 % $(NH_4)_2SO_4$ saturation by the addition of the solid salt. After stirring for 20 min the solution was centrifuged at 14 000 rpm and the precipitate taken up in a solution consisting of potassium phosphate (1×10^{-3} M)-versene (1×10^{-4} M)-glutathione (0.5 mg/ml), pH 7.7. The total amount of protein in this fraction was 480 mg as measured by ultraviolet absorption.[8] Incubations were carried out as described in Table 1. After incubation, the enzyme was denatured by heating at 100° for 2 min and the protein removed by centrifugation. The reaction mixtures were adjusted to pH 1–2 with 10 N HCl and hydrolyzed at 100° for 2 h with a condenser marble to minimize evaporation. The hydrolytic procedure removes the phosphate group from glucosamine-6-phosphate leaving glucosamine, which gives a greater color in the following colorimetric analysis. After neutralization with NaOH the reaction mixtures were analyzed for glucosamine by the acetic anhydride method of Ghosh *et al.*[9]

Experiment 2. Extraction was carried out in the same manner as described above but in this case $(NH_4)_2SO_4$ was first added to 20 % saturation and the resulting precipitate discarded. The supernatant was taken to 70 % saturation, the protein harvested by centrifugation and dissolved in the same solution

Table 1.

Drug addition (μmole)	Glucosamine synthesized (μmole)	% Inhibition
None	0.13	—
Salicylate (10)	0.13	0
» (25)	0.07	46
p-Hydroxybenzoate (10)	0.16	0
» (25)	0.16	0

Incubation mixtures contained the following (in μmoles): D-fructose-6-phosphate (25), L-glutamine (40), potassium phosphate pH 7.7 (80), versene (2.5), drug as indicated, enzyme (2 ml, 48 mg protein) and water to a volume of 4.73 ml. A control contained no drug plus boiled enzyme to give the amount of glucosamine-6-phosphate present in the enzyme solution itself. This value has been subtracted from the data reported. Incubation carried out for 2 h at 37°.

Table 2.

Drug addition (μmole)	Glucosamine synthesized (μmole)	% Inhibition of glucosamine-6-phosphate synthesis	% Inhibition PAPS synthesis and transfer *
None	0.10	—	
Salicylate (10)	0.08	20	
» (25)	0.06	40	
» (100)	0.00	100	
p-Hydroxybenzoate (10)	0.12	0	
» (50)	0.09	10	
Salicylate (100)			0
» (200)			0

Incubations for glucosamine-6-phosphate synthesis carried out as described in Table 1 except that the final volume was 4.23 ml.

* Taken from the data of Jacobson and Boström [7] for the effect of salicylate on the PAPS system from calf fetal cartilage.

as before. The results are presented in Table 2.

Attempts to study the effect of salicylate on the PAPS synthesizing and transfer system of the mouse tissue were not successful, perhaps due to the low activity of the final enzyme in this reaction sequence, *i.e.*, the enzyme transferring sulfate from PAPS to the acceptor, which in this case was *p*-nitrophenol. In order to present some comparison between the effects of salicylate on glucosamine-6-phosphate and PAPS synthesis in fetal tissue, the data in column four of Table 2 are taken from a previous report,[7] and shows the effect of this drug on the PAPS system isolated from calf fetal cartilage. The assay depends on the colorimetric determination of the *p*-nitrophenol remaining after sulfation has occurred, since *p*-nitrophenylsulfate produces no color in the method employed. A more detailed description of the experimental conditions is presented in the earlier report.

The data presented show that the synthesis of glucosamine-6-phosphate by L-glutamine-D-fructose-6-phosphate amino transferase prepared from mouse fetal tissue is inhibited by sodium salicylate, a potent anti-inflammatory drug, and one which also produces malformations in fetuses from drug-treated mothers. A structural isomer of salicylate, *p*-hydroxybenzoic acid, has no anti-inflammatory effect, does not produce fetal damage when given in the same concentrations as salicylate, has no effect on sulfate-incorporation into mucopolysaccharides and did not inhibit L-glutamine-D-fructose-6-phosphate amino transferase from mouse fetal tissue. These results are in agreement with those from experiments using the same enzyme prepared from bovine heart valves and calf fetal cartilage [7] and may also offer one explanation for the decrease in sulfate incorporation into mucopolysaccharides seen in heart valve slices on addition of salicylate to the medium.[10] The enzymatic synthesis of glucosamine-6-phosphate is an early irreversible [9] step in the biosynthetic pathway leading to the formation of the N-acetylgalactosamine moiety of chondroitin sulfate. An inhibition at this point may thereby decrease the amount of chondroitin sulfate synthesized due to a decreased amount of hexosamine precursor and sulfate acceptor.

Although no data are available for mouse fetal tissue, in contrast to its action on glucosamine-6-phosphate synthesis, sodium salicylate has no inhibitory effect on PAPS synthesis or sulfate transfer in bovine heart valves and calf fetal cartilage.[7]

The malformations observed in mouse embryos from salicylate-treated mothers were confined to vascular and skeletal tissues,[4,5]

both of which are known to contain acid mucopolysaccharides.[11] Since the rate of synthesis of mucopolysaccharides in embryonic tissues may play a role in their growth and differentiation,[12] a relationship may be inferred between the known teratogenic action of drugs such as salicylate [4-6] and cortisone [3] and their inhibitory effect on mucopolysaccharide synthesis as evidenced either by depression of sulfate incorporation or, as presented in this report, by depression of the synthesis of a key intermediate in mucopolysaccharide biosynthesis.

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1. Whitehouse, M. and Boström, H. *Biochem. Pharmacol.* **7** (1961) 135.
2. Whitehouse, M. and Boström, H. *Biochem. Pharmacol.* **11** (1962) 1175.
3. Larsson, K. S. *Acta Odontol. Scand.* **20** (1962) *Suppl.* **31**.
4. Larsson, K. S., Boström, H. and Ericson, B. *Acta Paediat.* **52** (1963) 36.
5. Larsson, K. S., Ericson, B. and Boström, H. *Acta Morphol. Neerl. Scand. In press.*
6. Larsson, K. S. and Boström, H. *Acta Paediat. In press.*
7. Jacobson, B. and Boström, H. *Biochim. Biophys. Acta*, **83** (1964) 152.
8. Warbourg, O. and Christian, W. *Biochem. Z.* **310** (1942) 384.
9. Ghosh, S., Blumenthal, H., Davidson, E. A. and Roseman, S. *J. Biol. Chem.* **235** (1960) 1265.
10. Boström, H., Moretti, A. and Whitehouse, M. *Biochim. Biophys. Acta* **74** (1963) 213.
11. Meyer, K., Davidson, E. A., Linker, A. and Hoffman, P. *Biochim. Biophys. Acta* **21** (1956) 506.
12. Balazs, A. and Holmgren, H. *Proc. Soc. Exptl. Biol. Med.* **72** (1949) 142.

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Mechanism of Oxidation of Inorganic Thiosulfate and Thiosulfate Esters in Mammals

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Thiosulfate is a metabolic product in higher animals [1] and is used as antidote against cyanide,[2] sulfur and nitrogen mustards.[3,4] A knowledge of its metabolism in higher animals is consequently of interest. Early investigators [5] demonstrated that thiosulfate injected into mammals is oxidized to sulfate, and Pirie [6] found that slices from certain rat tissues could accomplish this reaction although extracts from these tissues were practically inactive. Using thiosulfate labelled with ^{35}S in the outer and inner position, Skarzynski *et al.*[7] found that when thiosulfate was injected into rats most of the sulfate formed was derived from the inner sulfur atom, whereas the outer sulfur atom entered into tissue metabolism. No further details about the mechanism of thiosulfate oxidation in higher animals are available. However, certain microorganisms, for instance the sulfur bacteria, can also oxidize thiosulfate to sulfate, and the enzymatic mechanisms responsible for this reaction in *Thiobacillus thio-oxidans* have recently been elucidated by Peck.[8] In this case, thiosulfate is first reduced to sulfite by glutathione in a reaction catalyzed by the enzyme thiosulfate-reductase which was previously discovered in yeast by Kaji and McElroy.[9] The sulfite thus formed is then oxidized to sulfate in an AMP and phosphate dependent reaction. The aim of the present investigation was to study if the oxidation of thiosulfate to sulfate in rat liver takes place by the same mechanism as in bacteria.

The ability of rat liver slices to form sulfate from thiosulfate in an oxygen atmosphere as reported by Pirie [6] was first confirmed in the present investigation. It was necessary to carry out the reaction in oxygen, as otherwise the small amounts of sulfate found were barely detectable by the analytic technique used. An average value of 84.5 $\pm$ 10.2 (mean $\pm$ standard error of 8 determinations) μmoles of sulfate