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***p*-Ethylphenylsulphuric Acid in Goat Urine**

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Although it was suggested by Baumann in 1879 that *p*-ethylphenol might be formed in the animal body by the degradation of tyrosine, the presence of this substance was not reported until 1927, when Walbaum & Rosenthal (1927) and Pfau (1927), isolated it from the dried scent glands of the beaver. More recently, Lederer (1943, 1946) isolated *p*-ethylphenol from an extract of acid-hydrolyzed pregnant mare urine.

In the present work the isolation of *p*-ethylphenylsulphuric acid from urine as the potassium salt is reported for the first time. This substance was initially obtained from the urine of an ovariectomized goat, which had received large doses of progesterone and hexoestrol. Subsequently it was also isolated from the urine of a normal goat.

In view of the belief (Williams, 1947) that *p*-cresol is quantitatively the most important phenol in the urine of vertebrates, it is noteworthy that no clear-cut evidence has been obtained for the presence of *p*-cresyl sulphuric acid in the urine examined. The fact that the derivatives of *p*-ethylphenol, prepared from the hydrolysis product of the sulphate, required frequent recrystallization before constant melting points could be obtained, might suggest that the isolated substance was contaminated with

appreciable amounts of the sulphate of *p*-cresol or of other phenols. Nevertheless, the present work indicates that in the goat *p*-ethylphenylsulphuric acid is excreted in larger amount than *p*-cresylsulphuric acid.

EXPERIMENTAL

The experimental animals were mature ovariectomized goats on a diet of hay and a mixture of bran, linseed cake, oats and beans. For the purpose of lactation studies under the direction of Dr S. J. Folley at the National Institute for Research in Dairying, they received 1 mg. hexoestrol and 40 mg. progesterone daily. Dr Folley and Mr A. T. Cowie kindly arranged for the collection of urine, and for its dispatch to this laboratory.

The urine was collected quantitatively under butanol. Daily collections were stored in the refrigerator. The pooled 7-day collections received were worked up for conjugated substances. Normal goat urine was collected similarly from an animal receiving no treatment.

Extraction of urine

Urine (3.5 l.) from a treated goat was acidified to pH 3 with HCl, and rapidly extracted with butanol at low temperatures. The butanol extract was washed with $\frac{1}{3}$ -NaOH, and water, and evaporated to dryness under reduced pressure. The residue was dissolved in water, chilled to 0°,

acidified to pH 2 with HCl, and rapidly extracted with chilled ether. The ether-washed aqueous phase was made alkaline to litmus with NaOH, and extracted with butanol. After washing with water the butanol was evaporated *in vacuo*, leaving 5 g. of light brown solid.

Preparation of potassium and p-toluidine salts

The above-described solid was dissolved in a minimum of water, and an equal volume of saturated potassium acetate solution added. The crystalline precipitate formed was washed twice with half-saturated potassium acetate solution, and dissolved in 200 ml. butanol. After washing several times with water, the butanol was evaporated *in vacuo*, leaving 1.933 g. of nearly white crystals. After crystallization from 4% (v/v) aqueous acetone, crystals melting at 264–266° (corr.) were obtained.

The material thus obtained dissolved readily in water, and gave a test for sulphate after warming with HCl. Millon's reagent gave a positive reaction after heating several minutes at 100°. The Tollens' reaction for glucuronic acid was negative. N, P and halogens were absent.

After drying for several days *in vacuo* over P₂O₅, the following analytical results were obtained: C, 40.05, 40.09; H, 3.86, 3.79; S, 14.0, 13.9; K, 16.5, 16.8; *potassium ethylphenylsulphate* (C₈H₉SO₄K) requires C, 39.98; H, 3.78; S, 13.34; K, 16.27%.

0.154 g. of the above-described potassium salt, and 0.225 g. of *p*-toluidine hydrochloride were dissolved separately in 5 ml. lots of water. The two solutions were warmed to 50° and mixed. The precipitate of *p*-toluidine salt formed was recrystallized three times from water at 50°, giving a product melting at 164–166° (corr.) (decomp.). Mixed with a sample of authentic *p*-toluidine salt of *p*-ethylphenylsulphuric acid, there was no depression in melting point. Found: C, 58.25, 58.24; H, 6.02, 6.02. *p-Toluidine salt of p-ethylphenylsulphuric acid* (C₁₅H₁₉NO₄S) requires C, 58.24; H, 6.19. Heating above 50° caused marked decomposition of the *p*-toluidine salt of the sulphate.

Hydrolysis of the potassium salt

Hydrolysis was effected by boiling the potassium salt with approximately N-HCl for 20 min., cooling, and extracting with ether. The residue obtained on evaporation of the washed ether was distilled at 100° and 0.1 mm. to give a white crystalline solid. This had a strong phenolic smell and gave a blue colour, turning to dirty green, with ferric chloride solution.

Benzylation by the Schotten-Baumann method yielded a product which, after six recrystallizations from moist ethanol, melted at 59–60° (corr.). Mixed with authentic *p*-ethylphenyl benzoate (m.p. 60°, corr.) the m.p. was 58–60° (corr.). Mixed with authentic *p*-cresyl benzoate (m.p. 71°, corr.) the m.p. was 53°. Found: C, 79.64, 79.49; H, 6.06, 6.05. Calc. for *p*-ethylphenyl benzoate (C₁₆H₁₈O₂): C, 79.64; H, 6.23, for *p*-cresyl benzoate (C₁₄H₁₆O₂): C, 79.23; H, 5.70%.

The oxyacetic acid derivative of the ether-soluble hydrolysis product, after five recrystallizations, melted at 94–95° (corr.). Mixed with authentic *p*-ethylphenoxyacetic acid (m.p. 95–96°, corr.) the m.p. was 94–95° (corr.) and mixed with authentic *p*-methylphenoxyacetic acid (m.p. 136°, corr.) the m.p. was 97–98° (corr.). 5.106 mg. dissolved in water required 1.17 ml. 0.0236 N-NaOH giving an equivalent of 184.9. Calc. for *p*-ethylphenoxyacetic acid 180.2.

Normal goat urine

By the processes described above 5.4 l. normal goat urine gave 1.15 g. potassium salt. The *p*-toluidine salt prepared from this gave C, 58.46, 58.12; H, 6.11, 5.96. *p-Toluidine salt of p-ethylphenyl sulphuric acid* (C₁₅H₁₉NO₄S) requires C, 58.24; H, 6.19%. The benzoate obtained from the ether-soluble hydrolysis product, after six recrystallizations from ethanol, melted at 57° (corr.). Mixed with authentic *p*-ethylphenyl benzoate (m.p. 60°, corr.) the m.p. was 56–57° (corr.). After seven recrystallizations from water the oxyacetic acid melted at 95°; mixed with authentic *p*-ethylphenoxyacetic acid the m.p. was 94–95°.

SUMMARY

1. The isolation of *p*-ethylphenylsulphuric acid from animal sources, as the potassium salt, is reported for the first time.

2. The *p*-ethylphenylsulphuric acid was obtained from goat urine, in which it appears to be quantitatively the most important phenylsulphuric acid.

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