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Synthesis of Potassium Benzo(*a*)pyren-3-yl Sulfate

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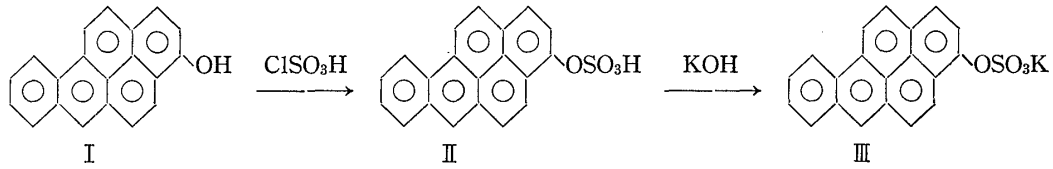
Benzo(*a*)pyren-3-yl hydrogen sulfate was synthesized from chlorosulfonic acid and benzo(*a*)pyren-3-ol, and crystallized as the potassium salt. It was hydrolyzed by aryl sulfatase. The UV, IR, and fluorescence spectra of the potassium salt are presented.

Keywords——potassium benzo(*a*)pyren-3-yl sulfate; IR spectrum; UV spectrum; fluorescence spectrum; enzymatic hydrolysis

Benzo(*a*)pyren-3-ol, one of the major metabolites of benzo(*a*)pyrene,[2,3] can be extracted, at least in part, as its sulfate conjugate.[4–10] Cohen *et al.*, in their study on the structure of benzo(*a*)pyrenyl hydrogen sulfate isolated from mammalian cell cultures fed with benzo(*a*)-pyrene, synthesized the sulfate for identification purposes by treatment of a sample of benzo-(*a*)pyren-3-ol with chlorosulfonic acid.[7] They found that the two compounds were identical with respect to UV and fluorescence spectra. It is possible, however, that the sample of benzo(*a*)pyren-3-ol used as the starting material had been contaminated with isomeric phenols, because this material had been prepared by incubating benzo(*a*)pyrene with rat liver homogenate.[8] It is known that the 9-ol,[9] 1-ol[11] and 7-ol[11] of benzo(*a*)pyrene can be formed by such treatment.

The present paper describes the synthesis and properties of a pure sample of potassium benzo(*a*)pyren-3-yl sulfate, starting from chemically synthesized benzo(*a*)pyren-3-ol.

Benzo(*a*)pyren-3-ol (I) was synthesized from anthracene by the method of Cook *et al.*[12] with some modification at the cyclization step.[13] The benzo(*a*)pyren-3-ol was treated with chlorosulfonic acid in dimethylaniline and the sulfate (II) was isolated as its potassium salt (III).



I II III

Chart 1

1) Location: *a*) *Maidashi 3-1-1, Higashi-ku, Fukuoka 812, Japan*; *b*) *Maidashi 3-1-1, Higashi-ku, Fukuoka 812, Japan*; *c*) Present address: *Section of Toxicology, Faculty of Pharmaceutical Sciences, Toyama Medical and Pharmaceutical University, 2630, Sugitani, Toyama 930-01, Japan.*
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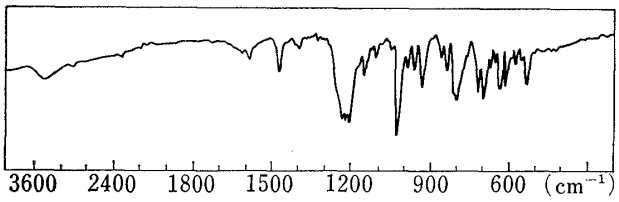

Fig. 1. IR Absorption Spectrum of III (KBr)

The results of elemental analysis (C and H) of this compound are in good accord with the structure III. The IR absorption spectrum (Fig. 1) showed strong peaks at 1245 and 1045 cm^{-1} assignable to a sulfate function. The presence of a sulfate linkage was supported by the observation that it was hydrolyzable with phenol sulfatase. The UV absorption spectrum of III, which was similar to that of Harper's sample,[5] was of a fully aromatic benzo(*a*)pyrenoid type (Table I). The excitation and fluorescence spectra of III and I are shown in Fig. 2. These results confirm that III is potassium benzo(*a*)pyren-3-yl sulfate.

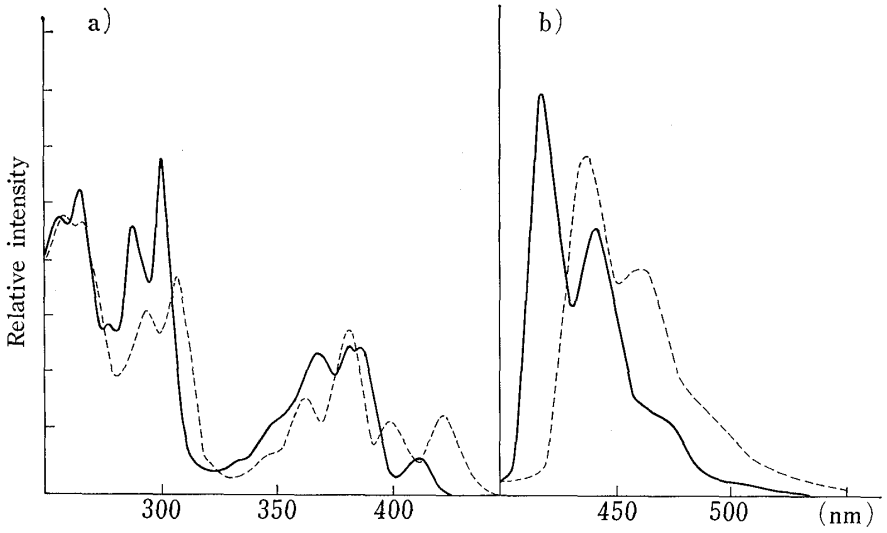

Fig. 2. Excitation and Fluorescence Spectra of III (——) and I (……)

Concentration : 1×10^{-7} M (in 50% EtOH)
a) Excitation spectra : III, at λ_{em}=414.5 nm, I, at λ_{em}=435 nm.
b) Fluorescence spectra : III, at λ_{ex}=298 nm, I, at λ_{ex}=298 nm.

Table I. UV Absorption Maxima and Molecular Extinction Coeffcients (in 50% EtOH)

Compound III		Benzo(*a*)pyren-3-ol (I)	
λ_{max} (nm)	log ε	λ_{max} (nm)	log ε
408	4.00	423	4.39
388	4.60	399	4.37
381	4.65	381	4.68
368	4.59	362	4.45
362	4.50	345	4.00
351	4.32	306	4.78
334	3.91	293	4.68
301	4.94	268	4.85
289	4.86	258	4.87
277	4.63	228	4.71
267	4.89		
265	4.88		
256	4.85		
226	4.71		
222	4.67		

Since conjugates of N-hydroxyacetylaminofluorene are the final carcinogenic metabolites,[14] it is clearly of interest to determine whether conjugates of benzo(*a*)pyren-3-ol possess mutagenic and/or carcinogenic activities. The chemical synthesis of these conjugates may consequently be useful for the identification of conjugated metabolites and also for examinations of mutagenicity and carcinogenicity.

Experimental

Melting points are uncorrected. IR spectra were recorded with a JASCO spectrometer, model DS-301, in potassium bromide disks. UV spectra were recorded with Shimadzu 200S spectrophotometer. Excitation and fluorescence spectra were recorded with a Shimadzu RT-502 machine.

Thin-Layer Chromatography (TLC)——Silica gel plates (silica gel G, Merck, 0.25 mm in thickness) were activated at 105° for 30 min. Small portions of the synthetic reaction mixture and enzymatic hydrolysis sample were spotted on a plate and developed with *n*-BuOH–AcOH–H_2O (35: 3: 5) (solvent A) or 5% MeOH in benzene (solvent B). The chromatograms were inspected under an ultraviolet lamp.

Chemical Synthesis of III——A solution of 0.33 g of I in about 5 ml of dimethylaniline was treated with 0.1 ml chlorosulfonic acid, and then the mixture was stirred mechanically for 30 min. The progress of the reaction was followed by TLC (solvent A). This sulfonation reaction was performed in a dark room, the temperature being maintained below 0°. The mixture was then made alkaline with conc. KOH solution (1: 1). Solid material was filtered off by suction and washed thoroughly with ether. The ester salt was extracted from the residue with hot alcohol (90%) and the alcoholic solution was filtered through a funnel. The solvent was evaporated to dryness *in vacuo*. This residue was crystallized from EtOH–H_2O (9: 1) and recrystallized from the same solvent to give a brownish-yellow powder, mp 268—270° (dec.). The yield was 25 mg. *Anal.* Calcd for $C_{20}H_{11}KO_4S$: C, 62.16; H, 2.87. Found: C, 62.11; H, 2.91. IR ν_{max}^{KBr} cm^{-1}: 1245, 1045 (sulfate). The UV absorption maxima and molecular extinction coefficient are listed in Table I.

Enzymatic Hydrolysis of III——III was incubated at 37° for 5 hr in 3.0 ml of 0.2 M acetate buffer (pH 5.0) with phenol sulfatase (Sigma 100 units) in a dark room. The liberated I was extracted 3 times with 5 ml of hexane–acetone (4: 1) by shaking for 5 min. The combined extracts were dried over anhyd. Na_2SO_4, and the solvent was evaporated off *in vacuo*. The residue was dissolved in a small amount of MeOH. This MeOH solution was subjected to TLC. This sample showed the same mobility as authentic I on TLC (solvent B).

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