SUMMARY

1. The squalene and cholesterol contents of the sebum from the backs of normal children and adults have been studied. It has been confirmed that the squalene content of sebum is lower and the cholesterol content higher in children than in adults.

2. A relationship between the sebaceous activity of a particular area of skin and the squalene and cholesterol content of the sebum derived therefrom has been demonstrated. It is considered that dilution of the surface-film lipids by lipids from the horny-layer cells significantly alters the composition of the 'sebum' in areas of skin with low sebaceous activity, e.g. most skin areas of the child and the arms and legs of adults. This apparent dilution could account for the observed differences between the squalene content of the 'sebum' from children and that of adults. To avoid this dilution effect, it is suggested that for studies of the composition of sebum the sample should be collected from the back.

3. The wide variations in the squalene content of sebum, both from day to day and between different individuals, has been confirmed. This is not due to epidermal contamination of the sample nor solely to variable rates of oxidation of squalene in different samples of sebum.

4. Claims that squalene can inactivate SH groups and inhibit certain enzymes have not been confirmed, and squalene does not appear to possess significant fungicidal or bactericidal properties. It can cause reversible depilation in the rabbit but not in the guinea pig, mouse or rat, and this property is not specific for squalene but is shared by a number of saturated and unsaturated lipids.

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Metabolism of Polycyclic Compounds

11. THE CONVERSION OF NAPHTHALENE INTO 2-HYDROXY-1-NAPHTHYL SULPHATE IN THE RABBIT*

By E. BOYLAND AND P. SIMS

Chester Beatty Research Institute, Institute of Cancer Research: Royal Cancer Hospital, Fulham Road, London, S.W. 3

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By the use of paper-chromatographic analysis, Corner & Young (1954, 1955), Sato, Yamada, Suzuki, Fukuyama & Yoshikawa (1956) and Solomon (1955) detected the presence of a metabolite of naphthalene in the urine of rats dosed with naphthalene, which gave the colour reactions of

1:2-dihydroxynaphthalene after the chromatograms had been treated with mineral acid. By injecting ³⁵S-labelled sodium sulphate into the dosed animals, the first two groups of workers were able to show by the use of radiochromatograms that the metabolite was probably a sulphuric ester of 1:2-dihydroxynaphthalene. Furthermore, Corner & Young (1955) detected 1:2-dihydroxynaphthalene

* Part 10: Boyland & Solomon (1956).

after the urines had been heated with acid, and Sato *et al.* (1956) found that the incubation of 1:2-dihydroxynaphthalene and ^{35}S -labelled sulphate with liver slices also yielded the new metabolite. All the above workers found that the metabolite was also produced when rats were treated with *trans*-1:2-dihydronaphthalene-1:2-diol. Corner & Young (1954) showed that the new metabolite, which must be either 2-hydroxy-1-naphthyl sulphate or 1-hydroxy-2-naphthyl sulphate, was also present in the urine of guinea pigs, mice and rabbits dosed with naphthalene and they detected free 1:2-dihydroxynaphthalene in the guinea-pig urine.

The present work describes the isolation of 2-hydroxy-1-naphthyl sulphate from the urine of rabbits dosed with naphthalene, which was identical with the synthetic ester prepared by the Elbs persulphate oxidation of 2-naphthol. Desai & Sethna (1951) isolated 1:2-dihydroxynaphthalene from this oxidation.

EXPERIMENTAL

1:2-Dihydroxynaphthalene. This was prepared by the reduction of 1:2-naphthaquinone with sodium dithionite by the method of Fieser & Fieser (1939). The crude product was purified by distillation in a microsublimation apparatus (180° at 12 mm.), to yield 1:2-dihydroxynaphthalene in yellowish needles, m.p. 102° . The diacetate separated from ethanol in prisms, m.p. 109° (Found: C, 68.8; H, 5.2. Calc. for $\text{C}_{10}\text{H}_8\text{O}_4$: C, 68.8; H, 4.95%). Fieser & Peters (1931) recorded m.p. 109.5° for this compound.

Potassium and sodium 2-hydroxy-1-naphthyl sulphate. 2-Naphthol (10 g.) and KOH (10 g.) in water (100 ml.) were cooled to 0° and a saturated aqueous solution of $\text{K}_2\text{S}_2\text{O}_8$ (20 g.) was added during 8 hr. with continuous stirring. The mixture was kept overnight at room temperature, the dark solid was filtered off and discarded, and the filtrate was adjusted to pH 4 with acetic acid. The solution was washed with ether (3×100 ml.) to remove unchanged 2-naphthol, and passed through a bed of charcoal (100 g., activated, British Drug Houses Ltd.) supported in a glass chromatography tube. The bed was washed with water (2 l.) to remove inorganic sulphate, with aqueous 2N- NH_3 soln. (1 l.), which removed coloured materials, and finally with methanol (1.5 l.) to elute the sulphuric ester. The methanolic eluate was evaporated to dryness under reduced pressure and the residue was recrystallized from aqueous ethanol to yield potassium 2-hydroxy-1-naphthyl sulphate (3.4 g.) in elongated plates (Found: C, 43.2; H, 2.8; S, 10.9; K, 13.9. $\text{C}_{10}\text{H}_7\text{O}_5\text{SK}$ requires C, 43.15; H, 2.5; S, 11.5; K, 14.05%).

When the potassium salt (1 g.) in 5N-HCl (5 ml.) was heated to 100° for 30 min. and cooled to 0° , 1:2-dihydroxynaphthalene (380 mg.) separated in glistening plates (m.p. $58\text{--}59^\circ$), which darkened on exposure to air. The crystals were dried at 100° at 0.2 mm. and recrystallized from light petroleum (b.p. $60\text{--}80^\circ$) in needles, m.p. and mixed m.p. $98\text{--}100^\circ$. The diacetate formed prisms from ethanol, m.p. and mixed m.p. 109° . The mother liquors from the hydrolysis were shown, by the use of BaCl_2 , to contain inorganic sulphate.

The potassium salt (2 g.) was dissolved in 5N- NaOH (10 ml.) and ethanol (50 ml.) was added. The solution was warmed for a few minutes and cooled to 0° , when a substance which appeared to be the disodium salt of 2-hydroxy-1-naphthyl sulphate separated, which formed silky needles from aqueous ethanol. This substance was hygroscopic and proved unsuitable for analysis. A concentrated aqueous solution of the salt was therefore acidified to pH 2 with conc. HCl, when sodium 2-hydroxy-1-naphthyl sulphate separated. From methanol-ether it formed plates (Found: C, 45.6; H, 2.9; S, 11.7; Na, 9.0. $\text{C}_{10}\text{H}_7\text{O}_5\text{SNa}$ requires C, 45.8; H, 2.7; S, 12.2; Na, 8.8%). The sodium salt was hydrolysed with 5N-HCl and the product was purified as before to yield 1:2-dihydroxynaphthalene, m.p. and mixed m.p. $100\text{--}101^\circ$.

Properties of 2-hydroxy-1-naphthyl sulphate on paper chromatograms. On descending chromatograms on Whatman no. 1 chromatography papers, the ester had R_F 0.48 when the papers were developed with *n*-butanol saturated with 2N- NH_3 soln., R_F 0.45 with the organic phase of a mixture of *n*-butanol-ethanol-water (17:3:20, by vol.) (Solomon, 1955), and R_F 0.36 with the organic phase of the mixture isobutanol-3N- $(\text{NH}_4)_2\text{CO}_3$ -3N- NH_3 soln. (4:3:3, by vol.) (Corner & Young, 1954). The ester appeared as a bright-violet fluorescent spot when the papers were examined under a Hanovia Chromatolite ultraviolet lamp fitted with a Chance OX7 filter. When the chromatograms were sprayed with diazotized *p*-nitroaniline (0.2% in 0.1N-HCl), diazotized sulphanilic acid (0.05% in 0.1N-HCl) or 2-chloro-4-nitrobenzenediazoniumnaphthalene-2-sulphonate (N.N.C.D. reagent; a saturated solution in 0.1N-HCl), followed in each case by aqueous 10% Na_2CO_3 , the ester developed little or no colour within 4 hr., but later pale-brown colours appeared. When the papers were sprayed with 2N-HCl and heated in an oven to 70° for 20 min., the ester spot gave immediate purple colours (characteristic of free 1:2-dihydroxynaphthalene) with each of the reagents.

Dosing of animals and collection of urine. Four rabbits (body weight approximately 2 kg.), maintained on oats, rat cake, bread and water, were each injected intraperitoneally on four consecutive days with a solution of naphthalene in arachis oil (5 ml. of 20%, w/v). The animals were housed in metabolism cages designed to allow the collection of urine separately from faeces. The urines were collected up to the sixth day and were stored at 0° .

Isolation experiments. The urine was centrifuged to remove solid matter, acidified (pH 4) with acetic acid and washed several times with ether. It was then passed through a bed of activated charcoal (500 g.), which was afterwards washed with water (4 l.) to remove urea and inorganic salts, and then with aqueous 2N- NH_3 soln. (1.5 l.). The naphthalene metabolites were finally eluted with methanol (2 l.), and the eluate was evaporated to dryness under reduced pressure to a brown gum.

A cellulose-powder column was prepared: a mixture of *n*-butanol-cyclohexane-water (13:8:1, by vol.) was added to cellulose powder (300 g., Whatman standard grade) to form a slurry, which was poured into a glass column (length 100 cm., diameter 8 cm.) fitted with a sintered disk as a support for the absorbant. The column was filled to the top with the solvent mixture and kept overnight to allow the cellulose powder to settle, after which solvent mixture (2 l.) was passed through the column at the rate of 100 ml./hr.

The gum containing the metabolites was dissolved in a little water and the solution was absorbed in cellulose powder (5 g.) which was packed in a layer on top of the cellulose in the column. The solvent mixture was passed through the column at 50 ml./hr., when a broad band which had a violet fluorescence under ultraviolet light was seen to form. The eluate was collected in 100 ml. fractions, which were separately evaporated to dryness under reduced pressure and examined by means of paper chromatography with the solvent systems already mentioned. The earlier fractions contained mainly *trans*-1:2-dihydronaphthalene-1:2-diol and were discarded. Later fractions, which corresponded to the elution of the fluorescent band, were evaporated to form light-brown gums or partially crystalline solids, all of which contained 1-naphthyl sulphate, together with the new metabolite. These gums were combined and dissolved in water (5 ml.) and 5*N*-NaOH (5 ml.) was added, followed by ethanol (50 ml.). The mixture was warmed until crystals began to separate, cooled to 0° and filtered. The filtrate was evaporated down to half volume on the steam bath and cooled to 0°, when more crystals separated. The combined solids were recrystallized from aqueous ethanol to yield a substance which was probably the disodium salt of 2-hydroxy-1-naphthyl sulphate as silky needles (980 mg., after drying at 100° and 0.2 mm., corresponding to about 2% of the naphthalene administered), which had an infrared spectrum (measured, as a mull in liquid paraffin, between 5 and 15 μ) identical with that of the substance obtained from the oxidation of 2-naphthol. It was converted as before into sodium 2-hydroxy-1-naphthyl sulphate (420 mg.), which formed plates from methanol-ether (Found: C, 45.4; H, 2.7; S, 12.0; Na, 9.0. Calc. for $C_{10}H_7O_2SNa$: C, 45.8; H, 2.7; S, 12.2; Na, 8.8%). The infrared spectrum (measured, as a mull in liquid paraffin, between 5 and 15 μ) was identical with that of the synthetic sodium salt, and the two salts had identical properties on paper chromatograms. When urines of the treated animals were examined on paper chromatograms with the solvent systems, the ester had somewhat higher R_f values than those obtained when the pure compounds were run (cf. Solomon, 1955; Corner & Young, 1954, 1955).

The ester was hydrolysed with 5*N*-HCl and the product was purified as before to yield 1:2-dihydroxynaphthalene, m.p. and mixed m.p. 101–102°. The diacetate formed prisms from ethanol, m.p. and mixed m.p. 108–109°. The mother liquors from the hydrolysis contained inorganic sulphate.

DISCUSSION

The structure of the new metabolite follows directly from its method of synthesis and from the fact that it gives 1:2-dihydroxynaphthalene and sulphuric acid on acid hydrolysis. The present work throws no light on the method of formation of the ester in the

body but the work of Corner & Young (1955) and of Sato *et al.* (1956) indicates that 1:2-dihydronaphthalene-1:2-diol is an intermediate in its formation.

The metabolic pathway of naphthalene therefore involves oxidation to 1:2-dihydronaphthalene-1:2-diol (II). The diol (II) is then dehydrogenated to 1:2-dihydroxynaphthalene (III) which is then conjugated to yield the sulphuric ester (IV).

SUMMARY

1. The isolation of 2-hydroxy-1-naphthyl sulphate from the urine of rabbits dosed with naphthalene is described.
2. The ester was synthesized by the persulphate oxidation of 2-naphthol.

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