The Isolation, Characterization and Structure of Uroterpenol, a Monoterpene from Human Urine

By A. P. WADE AND G. S. WILKINSON

The Endocrine Unit, The Liverpool Clinic, Liverpool 7

AND

F. M. DEAN AND A. W. PRICE

The Robert Robinson Laboratories, University of Liverpool

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Human urine is shown to contain, in a conjugated form, a compound of molecular formula $C_{10}H_{18}O_2$, which is named uroterpenol. Details are reported of the isolation procedure, the preparation of derivatives and the chemical and physical properties, together with a proof that the compound is *p*-menth-1-ene-8,9-diol.

The work reported in this paper was initiated by the finding of a large amount of a sulphuric acid chromogen in the urine of a 4½-year-old girl who showed signs of excessive androgen activity in the form of hirsutism. The spectrum of the sulphuric acid solution showed a maximum at 430 mμ similar to that shown by 5β-pregnane-3α,20α-diol (pregnanediol). The urine fraction containing this chromogen was also the fraction that would contain pregnanediol, but acetylation of the material and chromatography on alumina (Klopper, Michie & Brown, 1955) showed that the amount of pregnanediol present was normal. The sulphuric acid chromogen was therefore not pregnanediol. The amount of unknown substance excreted in the child's urine, expressed in terms of pregnanediol, varied between 8 and 20 mg./day. It was therefore important to determine the nature of this material, because of its possible connexion with hirsutism. The same material has been found in the urine of children suffering from congenital adrenal hyperplasia, as communicated in a preliminary report (Edwards & Wade, 1965).

EXPERIMENTAL AND RESULTS

Melting points are uncorrected.

Thin-layer chromatography. Silica gel G (E. Merck A.-G., Darmstadt, Germany) was used for all thin-layer chromatography. The solvent systems were mixtures of ethyl acetate and benzene, as follows: (1) 3:2 (v/v); (2) 2:3 (v/v); (3) 1:9 (v/v). Uroterpenol and its derivatives were detected by spraying with one of the following reagents, (a) and (b) being heated for 5 min. at 90–100°, (c) being used at room temperature: (a) phosphotungstic acid (15%, w/v) in ethanol, which gives a yellow spot turning blue on a white background; (b) acetic acid–ethanol–conc. H_2SO_4 –anisaldehyde (50:50:10:1, by vol.) (anisaldehyde reagent), which gives a pink spot turning green; (c) aqueous per-

manganate containing $KMnO_4$ (1%, w/v) and Na_2CO_3 (6%, w/v), giving a yellow spot on a pink background.

Gas-liquid chromatography. For analytical work, a Perkin–Elmer F11 instrument was used in conjunction with a capillary column (100 ft. long) packed with polyethylene adipate, at 120°, with N_2 at 8–10 lb./in.² as carrier gas. For attempted preparative separations a Wilkins Aerograph Autoprep A-700 was employed in conjunction with a flame ionization detector and a column (20 ft. long × 0.37 in. diam.) packed with Celite (45–60 mesh) with a 30% coating of SE33 and used at temperatures in the range 150–250° and with pressures of carrier N_2 in the range 15–25 lb./in.².

Spectra. Ultraviolet absorption was measured in a Unicam SP.500 spectrophotometer and infrared spectra were obtained from films or KBr disks with a Perkin–Elmer 125 spectrophotometer. 1H nuclear-magnetic-resonance (n.m.r.) spectra were measured with a Varian A-60 instrument. Mass spectra were determined with an MSE double-focusing spectrometer operated at 70 eV and by the direct-inlet technique.

Isolation of uroterpenol from urine. Estimation of uroterpenol in urine by the anisaldehyde reagent (Edwards & Wade, 1965) showed that individuals differed considerably in the amount excreted. Normal male urine containing at least 10 mg./24 hr. was used for the isolation of the compound. It was collected without preservative.

Originally, the isolation procedure consisted of: (1) extraction of conjugates from urine; (2) hydrolysis of glucuronides; (3) separation into ketones and non-ketones by using Girard reagent T; (4) chromatography of the non-ketones on Florisil and elution with chloroform containing methanol (2%, v/v); (5) chromatography on silica and elution with benzene containing ethyl acetate (40%, v/v); (6) partition chromatography on Celite with the system light petroleum (b.p. 80–100°)–methanol–water (25:24:1, by vol.); (7) purification by high-vacuum distillation. The compound was located at each step by thin-layer chromatography.

Subsequently the Girard separation and all column chromatography was replaced by partition between benzene and water with greatly increased efficiency and

convenience. The final method of isolation was as follows.

Urine (2l.) was adjusted to pH 3.0 with conc. HCl, $(\text{NH}_4)_2\text{SO}_4$ was added (50g./100ml.) and the conjugates were extracted into ether-ethanol (3:1, v/v) (2×0.5 vol.) (Edwards, Kellie & Wade, 1953). The combined extracts were filtered and evaporated to dryness under reduced pressure in a rotary evaporator. Ten such extracts were combined for enzyme hydrolysis, dissolved in 0.5M-acetate buffer, pH 4.0 (500ml.), and incubated with limpet β -glucuronidase (2g.; 2×10^6 units) for 18 hr. at 37° . The hydrolysate was extracted with ethyl acetate-ether (1:1, v/v) (2×3 vol.), and the combined extracts were washed with n -NaOH (500ml.), and water (3×300 ml.), dried with Na_2SO_4 and evaporated to dryness.

The residue was dissolved in benzene (200ml.) and the solution extracted thoroughly with water (3×600 ml.). The combined water fractions were extracted with methylene dichloride (2×900 ml.), which was dried and evaporated to dryness.

Final purification was by chromatography on thin layers of silica gel. Dissolved in benzene, the material was divided between ten plates (each 20 cm. $\times$ 20 cm.) and applied as a strip. The plates were developed in solvent system (1), and the compound was located by spraying a strip 0.5 cm. wide down the centre of each plate with one of the reagents. The compound was recovered from the appropriate area by shaking the silica with methanol-ethyl acetate (1:1, v/v). Rechromatography under the same conditions gave an eluate showing only a single spot when chromatographed. Evaporation of the eluent to dryness gave a pale-yellow gum, which could not be crystallized but which was employed for chemical work. For spectroscopy and other analytical purposes the material was distilled in a sublimation apparatus at $55\text{--}60^\circ/0.2$ mm., giving *uroterpenol* (I; R=H) as a colourless gum devoid of selective ultraviolet absorption but having $[\alpha]_D^{25} + 76 + 3^\circ$ (c 0.424 in CHCl_3) (Found C, 70.0; H, 10.6. $\text{C}_{10}\text{H}_{18}\text{O}_2$ requires C, 70.6; H, 10.7%). The chief features of the mass spectrum are sum-

(I)

(II)

(III)

(IV)

(V)

(VI)

(VII)

(VIII)

(IX)

(Xa)

(Xb)

(Xc)

(Xd)

(Xe)

(XI)

(XII)

(XIII)

marized in Table 1. This compound was unaffected by Girard reagent T or other carbonyl reagents, and was not attacked by NaBH_4 . It was stable in hot or cold aq. 2N-alkali and cold 2N-HCl, but was destroyed by heating in acid at 100° for 10 min. Its volatility was sufficient to engender considerable losses in recovery by evaporation and similar techniques.

Uroterpenol acetates. (i) To a benzene solution of uroterpenol (4.0 ml. containing 10–15 mg.) was added acetic anhydride (2.0 ml.) and a few drops of pyridine. The mixture was left overnight at 25° . Ice-cold water (9 ml.) was added and the derivative extracted with ethyl acetate (2×15 ml.). The extract was washed first with 2N-HCl,

then with saturated NaHCO_3 and water, dried with Na_2SO_4 and evaporated to dryness. The residual gum was purified by thin-layer chromatography with solvent system (2), and gave *uroterpenol monoacetate* (I; R=acetyl) as a pale-yellow gum, which could not be crystallized (Found: C, 68.3; H, 9.3. $\text{C}_{12}\text{H}_{20}\text{O}_3$ requires C, 67.9; H, 9.5%). The mass spectrum is described in Fig. 1 and Scheme 1. The acetate was reconverted into the diol by dissolving it in methanol (2 ml.), adding saturated aq. NaHCO_3 solution (0.2 ml.) and leaving the mixture overnight at 37° . After the addition of water (8 ml.), the product was isolated with ether (2×20 ml.) and identified as uroterpenol by chromatography and infrared spectroscopy.

Table 1. *Mass spectrum of uroterpenol*

The strongest peaks are indicated thus †. Metastable peaks appear at positions marked *.

Nominal mass	Assignments for monopositive ions	Transitions giving metastable peaks
(170)	P (not observed)	
152	P-H ₂ O	* 96.3
139†	P-CH ₂ (OH)	* 105.3
134	P-2H ₂ O	
123		
122		
121†	P-CH ₂ (OH)-H ₂ O	* 71.5
109	C ₈ H ₁₃	
107	C ₈ H ₁₁	
105	C ₈ H ₁₀	
95	C ₇ H ₁₁	
94	C ₇ H ₁₀	* 91.1
93	C ₇ H ₉	
92	C ₇ H ₈	* 89.1
91	Tropylium

Fig. 1. Mass spectrum of uroterpenol acetate (I; R=acetyl).

1-Acetyl-*x*-methylcyclohexenes. The oxidations described below were thought to yield an acetylmethylcyclohexene, and a number of such compounds were prepared for reference purposes. Conversion of 2-, 3- and 4-methylcyclohexanones (VIII) into the corresponding 1-ethynylcyclohexan-1-ols (IX) and thence into the related 1-acetyl-*x*-methylcyclohexenes (Xa-e) was effected by known methods (Rupe, 1931, 1933). Prepared in this way, 1-acetyl-4-methylcyclohex-1-ene (Xa) was shown by gas-liquid chromatography and n.m.r. methods to be pure, but this was not the case for the 3- and the 2-methyl isomers. According to Rupe (1931, 1933), fractional crystallization of the semicarbazones followed by hydrolysis suffices to separate 1-acetyl-3-methylcyclohex-1-ene (Xb) from 1-acetyl-5-methylcyclohex-1-ene (Xc), but we could obtain

Table 2. Mass spectrum of uroterpenol p-nitrobenzoate

Metastable peaks appear at positions marked *.		
Nominal mass	Assignments for monopositive ions	Transitions giving metastable peaks
(319)	P (not observed)	
304	P-CH ₃	
302	P-OH	
301	P-H ₂ O	
224	P-C ₇ H ₁₁	
168	O ₂ N·C ₆ H ₄ ·CO ₂ H ₂	
167	O ₂ N·C ₆ H ₄ ·CO ₂ H	
152	P-O ₂ N·C ₆ H ₄ ·CO ₂ H	
151	P-O ₂ N·C ₆ H ₄ ·CO ₂ H ₂	
150	O ₂ N·C ₆ H ₄ ·CO	
139	P-CH ₂ ·O·CO·C ₆ H ₄ ·NO ₂	
134	C ₁₀ H ₁₄	* 105.7 ↓
121	C ₉ H ₁₃	
119	C ₉ H ₁₁	
106	C ₈ H ₁₀	
105	C ₈ H ₉	
104	C ₈ H ₈	* 89.1 ↓
95	C ₇ H ₁₁	
94	C ₇ H ₁₀	
93	C ₇ H ₉	
92	C ₇ H ₈	
91	Tropylium	

Table 3. Retention times for isomeric acetylmethylcyclohexenes and -hexanes, obtained on analytical columns

Compound	Formula	Retention time (sec.)
1-Acetyl-2-methylcyclohexene	(Xd)	274
1-Acetyl-3-methylcyclohexene	(Xb)	394
1-Acetyl-4-methylcyclohexene	(Xa)	291
1-Acetyl-5-methylcyclohexene	(Xc)	291
1-Acetyl-6-methylcyclohexene	(Xe)	258
1-Acetyl-2-methylcyclohexane	(XI)	366
1-Acetyl-3-methylcyclohexane	(XI)	352
1-Acetyl-4-methylcyclohexane*	(VII)	216

* From natural or synthetic sources.

2-methylcyclohex-1-ene (Xd) was found to contain about 12% of 1-acetyl-6-methylcyclohex-1-ene (Xe), and again attempts to separate the isomers failed on the preparative scale. Retention times are listed in Table 3.

1-Acetyl-x-methylcyclohexanes (XI). Hydrogenation of 1-acetyl-4-methylcyclohex-1-ene (1g.) was completed in 15 min. by shaking the solution in methanol containing palladium-on-charcoal (10%, w/w) (0.1g.) under H₂ at ordinary temperature and pressure. Removal of the catalyst and solvent left 1-acetyl-4-methylcyclohexane (VII) as an oil (0.6g.), giving the 2,4-dinitrophenylhydrazones, which crystallized from methanol as deep-orange plates, m.p. 131–133°, giving the mass spectrum shown in Fig. 2 (Found: C, 56.1; H, 6.4. C₁₅H₂₀N₄O₄ requires C, 56.2; H, 6.3%), and the semicarbazone, which had m.p. 158–159° (literature 159° or 175°) (Found: C, 60.6; H, 9.6. Calc. for C₁₀H₁₉N₃O: C, 60.9; H, 9.7%).

Hydrogenation of the appropriate (mixtures of) olefinic isomers gave 1-acetyl-3-methylcyclohexane as an oil, yielding the semicarbazone, m.p. 165–166° (Found: C, 61.1; H, 9.9. Calc. for C₁₀H₁₉N₃O: C, 60.9; H, 9.7%). For this compound earlier reports give m.p. 174–175°.

A similar hydrogenation furnished 1-acetyl-2-methylcyclohexane, which supplied a semicarbazone, m.p. 171–173°, in agreement with previous reports (Found: C, 60.7; H, 9.7. Calc. for C₁₀H₁₉N₃O: C, 60.9; H, 9.7%). In gas-liquid-chromatographic analyses all three acetylcyclohexanes behaved as pure single substances. Under the conditions used the 2- and 3-methyl isomers behaved similarly whereas the 4-methyl isomer was widely different (see Table 3).

Oxidation of uroterpenol. (a) Production of formaldehyde. Uroterpenol (10–20 μg.) in acetic acid (0.1 ml.) was treated with 3 mm-HIO₄ (1.4 ml.) during 1 hr. and then 40% (w/v)

only the latter in a pure state by this method, what has before been described as the 3-methyl isomer now being shown by analytical gas-liquid chromatography and n.m.r. spectroscopy to be a mixture containing about 34% of the 5-methyl isomer. On the preparative scale, the separation of the two isomers by gas-liquid chromatography was unsatisfactory. Obtained similarly, 1-acetyl-

Fig. 2. Mass spectrum of 1-acetyl-4-methylcyclohexane 2,4-dinitrophenylhydrazone (derived from natural or synthetic sources).

SnCl_2 in 0.1N-HCl (0.4 ml.) was added. The solution (0.4 ml.) was placed in the outer ring of a Conway unit (Conway, 1957) and 0.2% (w/v) chromotropic acid solution in 30N- H_2SO_4 (0.8 ml.) in the inner. Distillation continued overnight. The chromotropic acid solution was then heated for 30 min. at 100° and the colour that developed was measured spectrophotometrically. The absorption maximum, at $570\text{ m}\mu$, was identical with that given by authentic formaldehyde, and quantitative estimations by this method indicated that 1 mol. of formaldehyde was formed/mol. of uroterpenol.

Confirmation of formaldehyde production was obtained by oxidizing uroterpenol (1.5 mg.) with HIO_4 , diluting the mixture with water and extracting it with chloroform. The aqueous phase was distilled into an ethanolic solution of dimesone (10%, w/v). The crystals, which formed slowly, were purified from aqueous methanol, giving formaldehyde methone, m.p. 187° (literature 189°), identified by infrared and mass spectroscopy.

(b) Production of ketones. After conducting oxidation of uroterpenol with HIO_4 by the method of Pontis & Fischer (1963) we extracted the products into methylene chloride, examined them chromatographically on silica plates with ethyl acetate-benzene solvent systems and found at least six constituents in the mixture produced. Oxidation with NaIO_4 by the method of Metcalfe (1963) or with sodium bismuthate by the method of Norymberski (1952) gave two products detectable chromatographically, but these appeared to be decomposed by attempts at isolation. Oxidation with *tert*-butyl chromate by the method of Oppenauer & Oberrauch (1949) as modified by Menini & Norymberski (1962) gave another, more stable, product resembling in general properties and chromatographic behaviour an acetylmethylcyclohexene, as follows. Uroterpenol (approx. 35 mg.) in carbon tetrachloride (0.5 ml.) and pyridine (0.5 ml.) was oxidized by the reagent

(0.5 ml.) for 18 hr. at 25° and the excess of reagent was destroyed by being shaken for 15 min. with a saturated solution of citric acid in ethanol (2 ml.). After the addition of aq. N- H_2SO_4 (5 ml.) the organic products were extracted into methylene chloride (10 ml.) and washed with dilute NaOH and water. Chromatography with solvent system (3) and sprays (a) or (c) gave one major band and several minor bands. Elution of the main band with ethyl acetate-methanol (1:1, v/v) and evaporation of the solvent left a small quantity of an oil, shown by gas-liquid chromatography (retention time 258 sec.) to differ from all the acetylmethylcyclohexenes except 1-acetyl-6-methylcyclohexene and by the infrared spectrum (ν_{max} , 1705 cm^{-1}) to differ from this also. On treatment with aqueous-ethanolic 2,4-dinitrophenylhydrazine the oil yielded 4-acetyl-1-methylcyclohexene (VI) as the 2,4-dinitrophenylhydrazone, which crystallized from aqueous methanol as prisms, m.p. $118\text{--}120^\circ$, identified by the mass spectrum (Fig. 3) and an accurate mass determination on the parent ion, which gave a value of 318.133669 ($\text{C}_{15}\text{H}_{18}\text{N}_4\text{O}_4$ requires 318.132796).

Dihydrouroterpenol. When a solution of uroterpenol (25 mg.) in methanol (5 ml.) containing palladium-on-charcoal (10%, w/w) was shaken under H_2 at $20^\circ/760\text{ mm.}$, absorption ceased after the uptake of 3.3 ml. of H_2 (calc. for 1 double bond, 3.5 ml.). Filtration and evaporation supplied dihydrouroterpenol as a gum characterized chromatographically and spectroscopically: in particular, the mass spectrum was related to that of uroterpenol in the requisite fashion, e.g. the most important fragments were of nominal masses 141 and 123, the latter being formed from the former as evidenced by a metastable peak at 107.4 (compare Table 1). The method used for uroterpenol afforded a *p*-nitrobenzoate, which did not crystallize. However, the mass spectrum was closely related to that of the uroterpenol ester, showing an appropriate series of significant bands shifted to higher nominal masses by

Fig. 3. Mass spectrum of 4-acetyl-1-methylcyclohex-1-ene 2,4-dinitrophenylhydrazone.

2 units. Moreover, the n.m.r. spectrum of the ester showed an absence of vinylic resonances and the presence of a four-proton multiplet at τ 1.85 (aromatic hydrogen), a two-proton band at τ 5.73 (side-chain methylene) and a three-proton band at τ 8.79 (side-chain methyl). But the band at τ 9.1 was more complex than the doublet appropriate to the ring methyl group, and so the reduced diol is probably a mixture of diastereoisomers, and the failure of the nitrobenzoate to crystallize is probably a consequence of this fact. Thin-layer chromatography failed to separate the isomers.

Oxidation of dihydrouroterpenol. Dihydrouroterpenol (20 mg.) was oxidized by *tert.*-butyl chromate as described for uroterpenol. Chromatography on silica showed that only one ketone was produced, and this was identified as 1-acetyl-4-methylcyclohexane by comparisons in gas-liquid chromatography with the authentic ketone. The 2,4-dinitrophenylhydrazone was identical with an authentic specimen according to mixed m.p. determinations, chromatographic behaviour on silica and mass-spectral fragmentation patterns (Fig. 2).

Structural elucidation. Uroterpenol has structure (I; R=H). It has the molecular formula $C_{10}H_{18}O_2$ determined analytically on the gummy parent and on its derivatives, notably the crystalline *p*-nitrobenzoate, and confirmed by the mass spectra, e.g. Table 1, considered in detail below. It contains no carbonyl group since it is unaffected by borohydrides or Girard reagents and it is transparent in the 6μ region of the infrared spectrum. It absorbs strongly in the hydroxyl region at 3470 cm^{-1} (KBr disk) and, in mild conditions, supplies a monoacetate (I; R=acetyl) showing carbonyl absorption at 1737 and hydroxyl absorption at 3470 cm^{-1} , the *p*-nitrobenzoate absorbing correspondingly at 1712 and 3470 cm^{-1} . Formed in rather more vigorous conditions, the diacetate possesses no hydroxylic absorption. Hence uroterpenol is a diol.

That uroterpenol is a 1,2-diol with one primary alcoholic

function is shown by the periodate oxidation giving formaldehyde and supported by the n.m.r. spectrum, which contains a two-proton band at τ 6.5 appropriate to the methylene group of a primary alcohol. In the monoacetate this band suffers the characteristic shift to τ 5.95 and in the *p*-nitrobenzoate to τ 5.62. The resistance of the second alcoholic function to esterification suggests that it might be tertiary, a view established by the absence of any other absorption near τ 6 and by the absence of the splitting that would have been produced by a proton on a carbon atom immediately adjacent. Thus the grouping (II) is present.

Quantitative micro-hydrogenation established the presence in uroterpenol of one double bond, and infrared absorption at 789 cm^{-1} (absent after hydrogenation) indicated that this is trisubstituted. One of the substituents is a methyl group as demanded by a three-proton band at τ 8.35 in the n.m.r. spectrum of the parent compound, which also shows that there is only one vinylic hydrogen atom (τ 4.6), this suffering an ill-defined splitting most easily understood as originating from an adjacent (ring) methylene group. The structure of uroterpenol must therefore include grouping (III), and this must include one ring to account for the hydrogen content of the molecule.

The n.m.r. spectra contained one more distinctive feature: a three-proton band appearing at τ 9.0 in the spectrum of the diol and at τ 8.75 in that of the *p*-nitrobenzoate, and this must correspond to a quaternary methyl group. This can most easily be incorporated by combining it with expression (II), so producing expression (IV), the clearest evidence for which is the mass spectra of the acetate (Scheme 1 and Fig. 1) and the *p*-nitrobenzoate (Table 2). Accurate mass determinations on the ion of nominal mass 117 in the spectrum of the acetate gave 117.055325, corresponding closely to the value 117.055164 calculated for the ion shown, this proving that a methyl group is indeed attached to the same carbon atom as both oxygen functions. The intensity of this ion is perhaps unusually high for one

containing the hydroxyl group, but that can be understood since the ion may readily be formulated as ions such as (V), which would undoubtedly have additional stability. A second feature of the mass spectra is the strong tendency for fission into fragments from C_7H_{11} to C_7H_7 , the first being the expected complement of the ion of nominal mass 117 and the others representing its expected descent into the tropylium ion. However, structure (I) is only one of several that satisfactorily combine expressions (III) and (IV) and the residue, methane.

Finally, uroterpenol was shown to belong to the *p*-menthane series. Glycol fissions of uroterpenol appeared to be complex when periodate or bismuthate were the reagents. However, oxidation by *tert*.-butyl chromate gave essentially one compound, chromatographic analysis of which suggested that it was the expected ketone (VI), but, because of the lack of material, this was isolated as the 2,4-dinitrophenylhydrazone and identified by the mass spectrum (Fig. 3) [which was very closely similar to that of the 2,4-dinitrophenylhydrazone of ketone (Xa)] and by accurate mass determinations on the parent ion. On the other hand, the oxidation of dihydrouroterpenol smoothly led to 1-acetyl-4-methylcyclohexane (VII), identified with authentic material by several independent methods, thus completing the proof that uroterpenol has structure (I; R=H).

DISCUSSION

So far as we are aware, uroterpenol is the first monoterpene to have been isolated from human urine and fully identified. It appears to be excreted as a conjugate with glucuronic acid since the unconjugated compound appears only after enzymic hydrolysis and no significant amount of the free compound is found in urine.

The finding of the compound in the urine of a child with hirsutism suggested a steroidal nature, but few reactions characteristic of a steroid could be demonstrated. When sufficient material had been accumulated and its n.m.r. spectrum determined, its non-steroidal nature became obvious and a detailed investigation then established that the aglycone is a diol (I; R=H) formally derived from *p*-menthane.

The isolation of a monoterpene from human urine poses a question of origin. The compound might be synthesized by the body, but there is no previous record of such a process. On the other hand, terpenes are commonly found in essential oils, extracts of citrus fruits and a variety of herbs and plants (Williams, 1959), and so it must be assumed that terpenes are continually being absorbed and metabolized by man. It is therefore significant that the structure of uroterpenol is closely related to that of limonene (XII), a monocyclic terpene especially plentiful in citrus fruits. Gray, Hellman, Lunnon & Weiner (1955) found in human urine, and also in the pulp and peel of citrus fruits, a substance they designated X_6 , which interfered with the estimation of aldosterone. The excretion of this substance, a non-steroid, was increased after

the ingestion of orange juice, but X_6 , which has been shown to be an $\alpha\beta$ -unsaturated ketone, is clearly different from uroterpenol. Holness & Gray (1958) suggested that X_6 might be a sesquiterpene, though no final conclusion was reached. Clearly, however, human urine is likely to contain numerous metabolites derived from terpenes, although we have made no further search for them.

Little is known in detail about the metabolism of terpenoid hydrocarbons. In rabbits, *p*-menthene (XIII) forms a conjugate with glucuronic acid, showing that oxidation can take place *in vivo* (Hämäläinen, 1912). It might be supposed therefore that limonene can be hydroxylated and eliminated from the body after conjugation with glucuronic acid. Preliminary evidence that this may occur has been obtained. The ingestion of an aqueous homogenate of whole lemon, shown chromatographically to contain limonene, led to a considerably raised excretion of uroterpenol. In contrast, the ingestion of concentrated orange juice (Ulster Vitamins Ltd.), in which limonene was not detected, caused no rise. (Extract of whole orange has not yet been examined.) It is therefore possible that urinary uroterpenol originates from dietary limonene.

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