reduction of the 9-ketone of **II** the to 9S alcohols of **VIII** and **IX**. Compound **VIII** arizes from the stereospecific reduction of the 6aS, 10aS isomer of **II** and **IX** results from the stereospecific reduction of the 6aR, 10aR isomer of **II**.

Compounds **III**, **IV** and **V** occurred in control cultures albeit in amounts $< 10\%$ of that found in viable culture samples (the amounts of the transformation products were determined by scanning thin layer densitometry and by integration of gasliquid chromatograms). The small amounts of **III**, **IV** and **V** formed in the controls probably resulted from air oxidation of **I**.

The carboxylic acids, **XIV** and **XV**, were isolated from a hydrocarbon (alkane) oxidizing culture, Nocardia salmonicolor ATCC 19149. Notice that the alkyl side chain is either 2 or 4 carbon atoms shorter than the alkyl side chain of the starting substrate (**II**). These acids may arize from a β-oxidation mechanism similar to that reported for the microbial metabolism of alkanes[6]. By analogy to alkane metabolism, **II** may be converted by hydroxylation of the side chain terminal methyl group ($C_{7'}$) into an intermediate primary alcohol which is subsequently oxidized to a terminal carboxylic acid. Consistent with this mechanism, we have detected small amounts of other carboxylic acids and have made tentative structure assignments based on high resolution mass spectral data. Based on these assignments, the microbiological oxidation of **II** by culture 19149 appears to lead to 2 series of carboxylic acids: one containing a hydroxyl at $C_{9'}$ and carboxylic acid side chains of 5, 7, and 9 carbon atoms, respectively; the other series having a carbonyl at $C_{9'}$ and carboxylic acid side chains of 5, 7, and 9 carbon atoms, respectively.

The reactions described in this study may represent just a few of the many reactions that can be catalyzed using microorganisms. It is likely that microorganisms that modify **I** and **II** will catalyze similar reactions with other cannabinoid substrates. In addition, many other metabolites were detected in our screening program that have not yet been isolated or identified. Considering the reactions demonstrated and the potential that remains, we believe that microorganisms will play an important role in the development of new and possibly therapeutically useful cannabinoids.

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Isolation of 2-S-cysteinyldopa and 2,5-S,S-dicysteinyldopa from the urine of patients with melanoma[1]

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Summary. Urine from patients with melanoma metastases is shown to contain, in addition to the previously described 5-S-cysteinyldopa (**I**), 2 closely related metabolites which have been isolated and identified as 2-S-cysteinyldopa (**II**) and 2,5-S,S-dicysteinyldopa (**III**).

The urinary excretion of 5-S-cysteinyldopa (**I**) has been the object of extensive investigations showing its significance for the characterization of the metabolic activity of normal and pathological melanocytes[3,4]. In the pigment cell, this amino acid is formed by 1,6-addition of cysteine to dopaquinone and is subsequently converted to phaeomelanin pigments by oxidation, cyclisation, and coupling[5]. Normally only minute amounts of 5-S-cysteinyldopa are present in the urine, whereas a markedly increased level of excretion is observed in the urine of patients harbouring malignant melanoma[6].

In further scrutiny of melanoma urine for related metabolites of biochemical and/or clinical significance, we have isolated 2 additional catechol amino acids, 2-S-cysteinyldopa (**II**) and 2,5-S,S-dicysteinyldopa (**III**) (figure).

In a typical experiment, 850 ml of urine, collected for 24 h from a patient with melanoma metastasis, was adjusted to pH 1 with 6 M HCl and after filtration was passed through a column (1.8×10 cm) of Dowex 50 W X4, 200–400 mesh, H^+ form. After a prolonged washing with 0.5 M HCl (1000 ml), the column was eluted with 2 M HCl at a flow rate of 50 ml/h; 26 fractions of 40 ml each were collected and examined for the presence of catechol amino acids, both spectrophotometrically in the range 220–350 nm and chromatographically on precoated cellulose plates (Merck) with n-propanol-1 M HCl (3:2, v/v)[7], using as detecting reagents a ninhydrin solution and 3% ethanolic $FeCl_3$.

Fractions 1–8 forming the major elution peak were pooled and evaporated to dryness under reduced pressure to give a colourless residue which was taken up in water and rechromatographed on a Dowex 50 W column (1.8×10 cm), equilibrated with 2 M HCl. Elution with the same acid solvent gave a major peak fraction (57 mg) corresponding to 5-S-cysteinyldopa (**I**) and a faster moving band with a very similar UV spectrum. Further purification of this minor fraction on Whatman 3 MM paper with n-butanol-

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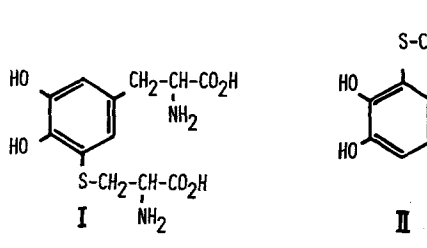
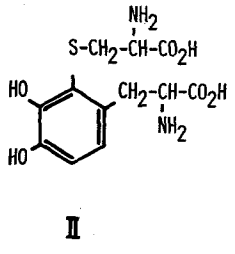
acetic acid–water (60:20:20, v/V) afforded 10 mg of 2-S-cysteinyldopa (**II**)[8] as an amorphous hygroscopic powder, λ_{max} (in 0.1 M HCl) 293 and 255 nm; NMR (D_2O) δ 3.14–3.70 (4H, m, $-CH_2-$), 4.06–4.36 (2H, m, –CH–), 6.89 and 6.99 (each 1H, ABq, J 8.3 Hz, ortho-coupled ArH)[9]. The structure of 2-S-cysteinyldopa was confirmed by a direct comparison of the spectral and chromatographic properties of the isolated product with those of an authentic sample.

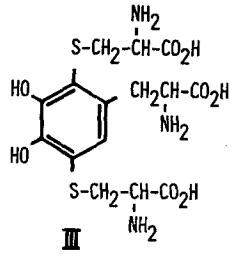
Fractions 14–25 contained a third catechol amino-acid. The fractions were evaporated to dryness and the residue was chromatographed on Whatman 3 MM paper using n-propanol-1 M HCl (3:2) as the eluent. Extraction with 0.1 M HCl of the major UV-quenching band (R_f 0.28) and evaporation of the extract gave 10 mg of the crude compound, which was further purified on a column (1.5 × 77 cm) of Sephadex G 25, developed with 0.1 M HCl. Fractions of 5 ml were collected and monitored by their E_{273}. Fractions 21–25 were combined and evaporated to dryness to give 3.6 mg of an amorphous colourless powder, λ_{max} 303 and 273 nm at pH 1 and 320 nm at pH 6.8 (phosphate buffer), NMR (D_2O)[9] δ 3.07–3.80 (6H, m, $-CH_2-$), 4.08–4.43 (3H, m, –CH–), and 7.10 (1H, s, isolated ArH), which was identified as 2,5-S,S-dicysteinyldopa (**III**) by comparison of the spectral and chromatographic properties with those of an authentic sample.



I



II



III

Indirect evidence for the natural occurrence of 2-S-cysteinyldopa (**II**) has recently been provided[10] by the isolation of trichochrome B from a melanoma urine, while the dicysteinyldopa (**III**) has previously been isolated from the reflecting spheres of the eyes of the alligator gar, Lepisosteus spatula[7].

Although a quantitative assessment of the excretory level of 2-S-cysteinyldopa and 2,5-S,S-dicysteinyldopa with respect to 5-S-cysteinyldopa in melanoma urine is out of the scope of the present work, the data so far available suggest that the relative ratio of **I**, **II** and **III** is approximately 15:3:1. This is consistent with the suggested metabolic origin of the cysteinyldopas and with recent biosynthetic experiments showing that in vitro the addition of cysteine to dopaquinone also leads to a mixture of **I**, **II** and **III** in a ratio very similar to that mentioned above[11,12]. If the same relative ratios of 5-S- and 2-S-cysteinyldopa and of 2,5-S,S-dicysteinyldopa can be demonstrated in quantitative experiments on melanoma urine, it will provide evidence that the dopaquinone-cysteine reaction taking place in the melanocytes proceeds more or less in the same way as in model experiments.

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Hyaluronic acid in elastic cartilage

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Summary. Bovine ear cartilage contains more hyaluronic acid than do hyaline cartilages of the same animal. Most of it is in the elastin-rich residue not extractable by 4 M guanidinium chloride where it is associated with chondroitin sulphate in low relative concentration and of lower molecular weight than in non-elastic cartilage residue.

The recent report[2] that cartilage proteoglycans can stimulate the fibrillation of elastin suggests an additional role for the glycosaminoglycans in elastic cartilages such as that of bovine ear. The glycosaminoglycan composition of ear cartilage differs only slightly from that of non-elastic cartilages of the same animal but the chondroitin sulphate chains are shorter[3] and there is a small but definite increase in the non-sulphated fraction[4]. This fraction includes hyaluronic acid which, following the initial report of Hardingham and Muir[5], is now known to cause the aggregation of cartilage proteoglycans even though present in very small quantities in these tissues[6]. In the present work these distinct features of ear cartilage glycosaminoglycans have been studied further to see whether they are associated with the presence of elastic fibres.

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