LETTERS TO THE EDITOR

The metabolism of butylated hydroxytoluene, (3,5-di-t-butyl-4-hydroxytoluene) in man

The urinary metabolites of [^{14}C]-3,5-di-t-butyl-4-hydroxytoluene (BHT) in man have been estimated by Daniel, Gage & others (1967), who found that over 50% of a 40 mg oral dose is excreted in the urine during the 24 h after dosing. The major metabolite (present to the extent of 35% of the dose) was later identified as a glucuronide of 4-carboxy-2-(1-carboxy-1-methylethyl)-6-(1-formyl-1-methylethyl)-

I

II
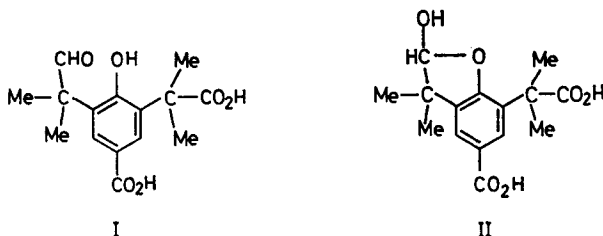
phenol (I) (Daniel, Gage & others, 1968). The agylcone was isolated as a hydrate of the dimethyl ester. Only 3% of the dose was eliminated as 3,5-di-t-butyl-4-hydroxybenzoic acid (BHT–COOH) and its glucuronide.

The oxidation of the t-butyl group has also been reported in rabbits given t-butylbenzene (Robinson & Williams, 1955) or BHT (Dacre, 1961). However, no t-butyl oxidation occurs with the t-butylcyclohexanones in the rat (Cheo, Elliot & Tao, 1967). Also Ladomery, Ryan & Wright (1967) were unable to demonstrate it with BHT in the rat and rabbit. It appears that when metabolic pathways other than oxidation of a t-butyl group are available such pathways predominate. It was therefore considered surprising that (I) was produced in such high yield in human urine. Investigations were made to verify the identity of this metabolite and determine the nature of the hydrate.

A group of 8 men each received 100 mg of BHT on two occasions with a 4 day interval. Urine was collected for 24 h after BHT administration and subjected to a work-up procedure identical to that employed by Daniel & others (1968), except that the DEAE cellulose column was omitted. The crude glucuronide gum was hydrolysed with 2M sodium hydroxide for 0·5 h and then continuously extracted with ether twice; once at pH 6 and again at pH 3. The metabolites present in the ether extracts were separated and identified using thin-layer chromatography (Holder, Ryan & others, 1970). Only BHT–COOH was detected in the first ether extract while the only component in the second extract to give a positive reaction with Gibb's reagent was identified as benzoylglycine (identified by infrared and nuclear magnetic resonance spectroscopy and melting point).

Subsequent urinary analyses were made in duplicate on the pooled 24 h urine of two adults each given 1·0 g of BHT. The urine was adjusted to pH 6 and extracted continuously with ether. A second extraction was made after further adjustment of the acidity to pH 2. Thin-layer chromatography of each extract showed the presence of a very polar compound which gave a positive test with naphthoresorcinol, and disappeared after β-glucuronidase or acid hydrolysis. Since the only compound present in significant amounts in the hydrolysed extract was BHT–COOH,

the polar component of the second extract was most probably BHT–COOH ester glucuronide. The carboxylic acid and its ester glucuronide were the only major metabolites detected in human urine.

It is rather surprising that these were the only metabolites present. It was expected that if the metabolite (I) were the major metabolite in urine, it would have been easily detected and isolated. However three separate attempts, using doses comparable and greater than those used by Daniel & others (1968) failed. The evidence for structure (I) is incomplete as it is based almost entirely on accurate mass measurements of a molecular ion peak at m/e 322 for the dimethyl ester. The specific radioactivity of the metabolite (a radiolabelled compound had been fed) and details of its infrared spectrum were not given. Furthermore, although some nmr data are given no signals were assigned to the aldehydic and phenolic protons, and D_2O exchange was not carried out. The absence of the aldehydic proton was ascribed to the formation of a stable hydrate, presumably the *gem*-diol of the aldehydic carbonyl. In general, diols obtained from aldehydes are unstable compounds only obtainable when the diol is stabilized by hydrogen bonding of the hydroxyl protons as with glyoxyllic acid, or by relief of dipole interactions as with trichloroacetaldehyde. For (I) there is no obvious driving force for such a reaction.

The cyclic hemiacetal (II) was considered as an alternative structure for Daniel's metabolite. However, the evidence presented is not sufficient since the nmr spectrum should show signals for one exchangeable hydroxyl proton and one methine proton. The signal at 4·6 τ (Daniel & others, 1968) could be assigned to the latter, but the absence of the hydroxyl signal remains. Furthermore since hemiacetals are usually too unstable to be isolated as such we consider structure (II) as unlikely.

In view of the foregoing, the occurrence of (I) as a BHT metabolite in human urine requires further verification. The postulated metabolite is unusual in that two alkyl groups have been completely oxidized to the carboxylic acid while the third is oxidized to the aldehyde. That oxidation of the t-butyl group proceeds beyond the alcohol is surprising, particularly in view of the results of Robinson & Williams (1955) who showed that t-butylbenzene is oxidized to 2-phenyl-2-methylpropanol only.

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