Tetrahedron Letters No. 24, pp I88I - I884, I975. Pergamon Press. Printed in Great Britain.

HYDROXYLATION OF CEDROL BY RABBITS

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(Received in France 2I April I975; received in UK for publication 23 April I975.

Scents and flavours can modify behaviour. This is of course usually taken as indicative of an action on chemoreceptors (chemotaxis, olfaction, gustation), and of subsequent neurological mechanisms. That this needs not always be the case is shown by the studies of Vesell, Wade, Hashimoto and coll. (1). Inhalation of cedrol 1 or α-cedrene 2 (or, initially the cedar-wood shavings containing them) shortens the duration of sleep induced in animals by barbiturates, by increasing the efficiency of the detoxification mechanisms occuring in the liver. Such induction of hepatic biochemical activity is known : hydroxylation of substrates can be accelerated by simultaneous, or prior, administration of other substrates (2).

As a first step in the study of some problems of behaviour chemistry, we have studied the products formed by feeding cedrol to rabbits, to check whether induction of detoxification of barbiturates was accompanied by oxidation of the inducer, or simply by conjugation. As we show below, both occur, and the oxidation of cedrol proceeds at a distance of the hydroxyl group, on a saturated, non-activated carbon atom.

METHODS:

"Fauve de Bourgogne" rabbits, weighing about 3 kg each, were starved for 36 hr before experiment. Natural cedrol (1 g) suspended in a 1 % methylcellulose mucilage (20 ml) followed by about 25 ml of water was administered to each unanaesthesized rabbit by stomach tubing (3). The urine collected at 24 hr, 48 hr and 96 hr after administration was acidified to pH 4,5 and hydrolyzed at 37°C for 24 hr with 6 ml of snail (D-glucuronide)-glucuronidase ("Suc d'Helix pomatia" of Industrie Biologique Française).

Silica-gel column chromatography of the ether extract gave :
- 50 mg of cedrol (probably excreted as glucuronides): yield 5 %,
-120 mg of a mixture of alcohols A: yield 12 %,
-350 mg of a mixture of diols B:yield 35%.

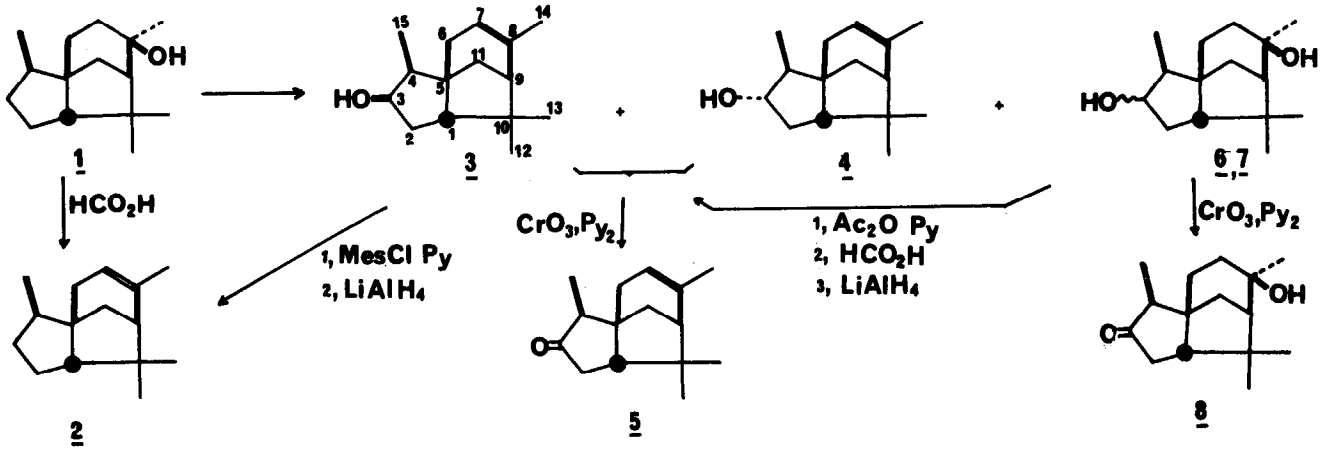
RESULTS:

The alcohol fraction A contained two alcohols, separable by further silica gel column chromatography. They were assigned structures 3 and 4, suggested by their n.m.r. spectra and proved by the reactions summarized in Table 1, which correlate them with cedr-7-ene 2. The location at C-3 of the new hydroxy group was proved by base-catalyzed deuteration of ketone 5 (4), to give a trideutero-ketone (m.s.), the n.m.r. spectrum of which now shows a singlet for the signal due to the C-15 methyl group : a deuterium atom must now be present at C-4, which places unambiguously the keto group at C-3. The configurations, 3-R 3 assigned to the less polar alcohol, and 3-S 4 to the more polar one, are required to explain the relative intensities of the paramagnetic shifts induced by $Eu(fod)_3$ (5) on the signals of the methyl groups in their n.m.r. spectra : in 3, the hydroxy group must be situated nearer the C-15 methyl group - and in 4 it must be situated nearer one of the methyl groups at C-10, as these show the largest paramagnetic shifts. Structure 3 is identical with that proposed for α-isobiotol (6). This was confirmed by comparison of n.m.r. and i.r. spectra, and of the rotatory power of 3 with α-isobiotol, and of 5 with α-isobiotone.

The diol fraction B could not be separated by silica gel column chromatography, but gave, upon dehydration, the alcohols 3 and 4. This, when considered with the spectral properties of the mixture B, or of the homogeneous ketone 8 obtained by dehydration, proves the structures 6 and 7. A 3α-alcohol of this structure has been isolated from the oxidation of cedrol with Aspergillus niger (7). We have not been able so far to compare samples.

DISCUSSION:

Elimination of a tertiary alcohol, such as cedrol, from an animal as its glucuronide is probably made difficult by steric hindrance. To facilitate this elimination, the organism preferentially forms conjugates by additional functionalization, for example by epoxidation of a double bond followed by hydration, by reduction of a ketone, by demethylation of a methoxy group or by hydroxylation of an aromatic carbon atom or a non-activated saturated carbon atom etc... (8). In the case of cedrol, the hydroxylation of a non-activated saturated carbon atom is the only possible pathway. In the case of the metabolic clearance of the barbiturates, especially the pento- and hexo-barbitals,



Alcohol 3 H_3C-15: 0.9 (d, 6 Hz) ; H_3C-12 & H_3C-13: 0.96 & 1.05 (s) ; H_3C-14: 1.7 (m) ; H C-3: 4.32 (q, 4.5 Hz) ; H C-7: 5.23 (m). $\Delta\delta^{n=1}_{Eu(III)}$: 3 & 3.5 for H_3C-12 & H_3C-13 ; 15 for H_3C-15. Liquid. $(\alpha)_D$ -65°. Less polar. 3%/cedrol administered.

Alcohol 4 H_3C-15: 0.97 (d, 6 Hz) ; H_3C-12 & H_3C-13: 1 & 1.05 (s) ; H_3C-14: 1.68 (m) ; H C-3: 3.66 (m) ; H C-7: 5.20 (m). $\Delta\delta^{n=1}_{Eu(III)}$: 2.25 & 4.25 for H_3C-12 & H_3C-13 and 11.5 for H_3C-15. m.p. 103-106°. $(\alpha)_D$ -53°. More polar. 9%/cedrol administered.

Ketone 5 H_3C-15: 1 (d, 6.5 Hz) ; H_3C-12 & H_3C-13: 1 & 1.1 (s) ; H_3C-14: 1.7 ; H C-7: 5.25 (m). MS. $M^{\cdot+}$ = 218 ($C_{15}H_{22}O$). After deuteration H_3C-15: 0.95 (s, 3H) ; H_3C-12 & H_3C-13: 0.95 & 1.1 (s) ; H_3C-14: 1.7 (m) ; H C-7 : 5.25 (m). MS. $M^{\cdot+}$ = 221 ($C_{15}H_{19}D_3O$). $(\alpha)_D$ -78°.

Ketone 8 H_3C-15: 1 (d, 6.5 Hz) ; H_3C-12 & H_3C-13: 1.02 & 1.3 (s) ; H_3C-14: 1.4 (s).

Note: $(\alpha)_D$ were recorded on a Perkin-Elmer-141 polarimeter, mass spectra on a LKB-9000 spectrometer and NMR on a Perkin-Elmer-12B spectrometer. Chemical shifts are reported in δ (ppm) values from internal tetramethylsilane standard.

the functionalization is also obtained by hydroxylation on a non-activated carbon-atom [9]. This probably explains the acceleration of elimination of barbiturates by cedrol in that their functionalization is brought about by liver microsomal enzymes possibly already employed in the hydroxylation of cedrol, and induced by this substance.

This hypothesis is confirmed by the observation that treatment of the rabbits with pentobarbital (3 mg/kg/day, 3 days) prior to the administration of cedrol leads to a marked increase in the proportion of cedrol hydroxylated at C-3 (60-70 % instead of 40-50 %).

We thank Professor J. Schwartz and Dr. F. Miesch (Institut de Pharmacologie et de Médecine Expérimentale, Faculté de Médecine, Université Louis Pasteur, Strasbourg) for their gracious help with the biological part of this work.

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