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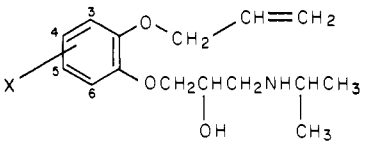
Communications to the Editor

Aromatic Hydroxylation of β-Adrenergic Antagonists. 4- and 5-Hydroxylation of 1-(Isopropylamino)-3-[2-(allyloxy)phenoxy]-2-propanol (Oxprenolol)

Sir:

Oxprenolol[1] [1-(isopropylamino)-3-[2-(allyloxy)phenoxy]-2-propanol, **1**] is an important aryloxypropanolamine β-adrenergic antagonist useful in the treatment of a variety of cardiovascular disorders. Like closely related propranolol [1-(isopropylamino)-3-(1-naphthyloxy)-2-propanol], oxprenolol is metabolized by hydroxylation of the aromatic ring and by oxidation of the propanolamine side chain, as well as by glucuronidation.[2-4] A possible glucuronide conjugate of oxprenolol has been isolated from rats.[2] A different conjugate was identified in human urine, which is subject to aqueous acid hydrolysis but not to β-glucuronidase.[4]

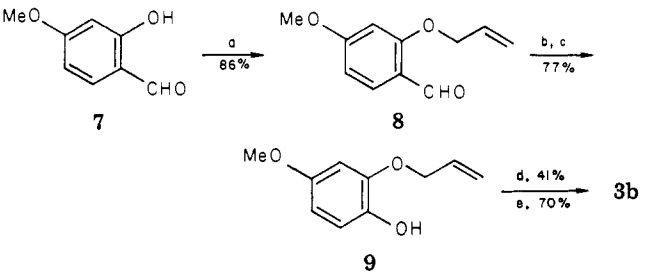


1, X = H
2, X = OH
3a, X = 4-OH
b, X = 4-OMe
4a, X = 5-OH
b, X = 5-OMe
5a, X = 3-OH
b, X = 3-OMe
6a, X = 6-OH
b, X = 6-OMe

The metabolite (**2**) from human urine (after treatment of the conjugate with aqueous 1 N HCl) was thought to be a phenol, based on mass spectral measurements on it and on the product formed by treatment of it with CH_2N_2.[4] The trifluoroacetyl (TFA) derivative of the phenolic metabolite from rats gave a single GC peak. Garteiz[2] suggested that the hydroxylated metabolite in rats, and thus probably in man, is 5-hydroxyoxprenolol (**4a**) rather than 4-hydroxyoxprenolol (**3a**), due to greater π-electron density of the allyl group[5] para to position 5. We set out to determine the structure of this metabolite, which was inferred on chemical rationale which might not be applicable to the enzymatically mediated metabolic processes. In this communication we report that both 4- and 5-hydroxylation of oxprenolol occurs in the rat in vivo and in man.

The high degree of symmetry of the oxprenolol molecule suggested to us that working from only limited amounts of metabolite toward a chemical proof of structure could prove tortuous, at best. We therefore chose to synthesize derivatives of the isomeric hydroxylated oxprenolols, where the location of the additional oxygen-bearing substituent would be known with certainty. The isomeric ring-substituted methoxyprenolols **3b–6b**[6] were prepared from the known isomeric 3-, 4-, 5-, and 6-methoxysalicylaldehydes. For example, 4-methoxysalicylaldehyde (**7**) was converted to 4-methoxyoxprenolol (**3b**, Scheme I) in 18% overall yield (four steps). The synthesis utilized the phenolic hydroxyl group of the salicylaldehyde as the position of the allyloxy substituent, followed by conversion of the aldehyde group to a phenol by Baeyer–Villiger oxidation. The allyloxy substituent was stable (not converted to an epoxide) under Baeyer–Villiger conditions, even in the presence of excess peracid. From the resulting new phenolic hydroxyl group, the propanolamine side chain was elaborated by O-alkylation with epichlorohydrin, followed by epoxide opening with isopropylamine. Identical processes afforded isomeric methoxyoxprenolols **4b–6b** in 15–19% overall yield. Starting materials were commercially available 5- and 3-methoxysalicylaldehyde and 6-methoxysalicylaldehyde prepared by the method of Hirose.[7]

Scheme I[a]



[a] Step a, allyl bromide-K_2CO_3 in DMF, 25 °C; b, *m*-Cl-$C_6H_4CO_3H$; c, NaOH-H_2O; d, epichlorohydrin-K_2CO_3 in acetone; e, *i*-$PrNH_2$.

The isomeric methoxyoxprenolols **3b–6b** were separated by GC as their TFA derivatives[2,8] formed by treatment with trifluoroacetic anhydride at 60 °C for 15 min. GC retention times were for **1**·TFA (oxprenolol) 5.7 min, **3b**·TFA (4-OMe) 13.1 min, **4b**·TFA (5-OMe) 11.8 min, **5b**·TFA (3-OMe) 9.6 min, and **6b**·TFA (6-OMe) 10.5 min.[9]

Metabolites were obtained by collection of 24-h urines from six male Sprague–Dawley rats administered oxprenolol 20 mg kg^{-1} ip. The combined urines (40 mL) were incubated with 9200 units of β-glucuronidase (Sigma) at 37 °C overnight, pH 5.0 (HOAc–NaOAc). Samples were adjusted to pH 9.2 (Na_2CO_3) and extracted with 2 × 50 mL of EtOAc. An aliquot (one-quarter of the total) of the evaporated EtOAc extract was treated with excess CH_2N_2 in Et_2O–MeOH (10:1), 6 h, and then subjected to trifluoroacetylation. Methylation was tried by several less successful methods, including MeI–Ag_2O–DMF and dimethyl sulfate–base. Diazomethane in the presence of Et_2O–MeOH proved to be most efficient. The reaction time was important, since overmethylation does occur with

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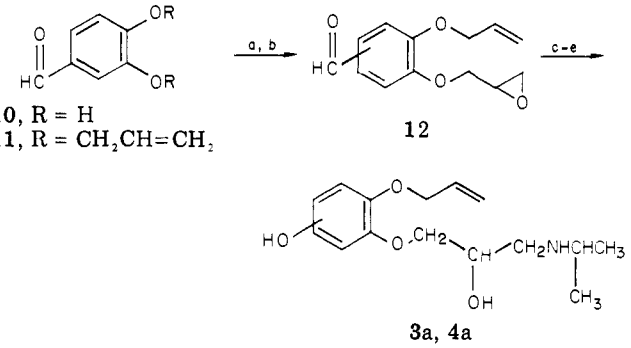
Scheme II[a]



10, R = H
11, R = $CH_2CH{=}CH_2$

12

3a, 4a

[a] Step a, NBS, wet dioxane; b, NaOH-H_2O; c, *m*-Cl-$C_6H_4CO_3H$; d, NaOH-H_2O; e, *i*-$PrNH_2$.

increased length of exposure to CH_2N_2.

A mixture of 4- and 5-hydroxyoxprenolol (**3a** and **4a**) was prepared from 3,4-dihydroxybenzaldehyde (**10**, Scheme II) in order to study the methylation conditions. Diallyl ether **11** was converted to a mixture of epoxides **12** by treatment with 1 equiv of *N*-bromosuccinimide, followed by NaOH. Baeyer–Villiger oxidation and hydrolysis followed by treatment with *i*-$PrNH_2$ afforded a mixture of **3a** and **4a**. After methylation, the mixture was shown by GC (TFA derivatives) to be approximately a 1:1 mixture of **3b** and **4b**.

Determination of metabolites was done by GC–CIMS monitoring *m/e* 488 (M + 1) and/or 374 (M + 1 – CF_3COOH)[10] at the appropriate retention times. By monitoring the total ion current at these masses, a ratio of approximately 1:4 of 5-methoxy- to 4-methoxyoxprenolol (**4b** to **3b**) as TFA derivatives was determined. No 3- or 6-methoxyoxprenolol (**5b** and **6b**) was found.

A 6-h human urine sample of one subject administered (orally) a mixture of 63 mg of oxprenolol-d_6[11] and -d_0 (1:1) was sequentially subjected to hydrolysis with aqueous 1 N HCl at 37 °C for 12 h and with β-glucuronidase (18 000 units, overnight, pH 5.0). From an EtOAc extract obtained identically with the EtOAc extract from rat urine, approximately a 1:4 mixture of 5- and 4-methoxyoxprenolol (**4b** and **3b**) was found.

These data confirm the formation of 4- and 5-hydroxyoxprenolol as important pathways of aromatic hydroxylation of oxprenolol in the rat and in man. Under these conditions neither 3- nor 6-hydroxylation was noted, although the absence of these potential metabolites cannot be completely assured. If formed, they may have been present in such small amounts that they are not detected by these analyses or possibly could be more slowly methylated by CH_2N_2 and thus not detected.

The confirmation of the presence of hydroxylated oxprenolol metabolites coupled with the observation that some of the pharmacological effects of oxprenolol in man are not well correlated with blood levels of oxprenolol[12] could suggest that these hydroxylated metabolites may contribute to observed effects of oxprenolol. Synthesis of sufficient quantities of these hydroxylated oxprenolols is underway to allow investigation of this possibility. Mechanistic aspects of this hydroxylation process are also under study.

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References and Notes

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Plant Antitumor Agents. 17. Structural Requirements for Antineoplastic Activity in Quassinoids[1]

Sir:

The initial finding[2] that the novel quassinoid holacanthone (**1**) had antineoplastic activity prompted an extensive search for other active quassinoids[3-8] with the result that some of the structural requirements for antineoplastic activity, particularly in the P-388 mouse leukemia system, are known. These have been recently reviewed.[9,10] It is well established that the Δ^3-2-oxo moiety in ring A, the lactone moiety in ring D, and ester groups at C-6 and/or C-15 are required for biological activity.[7-10] However, the role of the C-1, C-11, and C-12 hydroxyl groups and the epoxymethano bridge between C-8 and C-11 in most quassinoids or between C-8 and C-13 as in the bruceantin (**2**) series is unknown. This information is vital in planning the total synthesis of active quassinoids or analogues. We wish to present recent studies which indicate the absolute necessity of the epoxymethano bridge and the desirability of the C-1 and C-12 hydroxyl moieties for biological activity.

6α-Senecioyloxychaparrinone (**3**) [mp 254–257 °C dec; $[\alpha]^{22}_D$ +203.8° (*c* 0.210, MeOH); IR ($CHCl_3$) 1740 (δ-lactone), 1715 (α,β-unsaturated ester), 1680 (α,β-unsaturated ketone) cm^{-1}; UV (MeOH) 228 nm (log ε 4.34); ^1H NMR ($CDCl_3$) δ 1.29 (3 H, s, C-10), 1.93, 2.20 (6 H, s, C-4′, C-5′), 2.74 (1 H, s, C-9), 3.66, 4.16 (2 H, d, *J* = 8 Hz, CH_2O),

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