Model Studies with Enzyme Inhibitors. Addition of Nucleophiles to Conjugated Allenic 3-Oxo-5,10-Secosteroids

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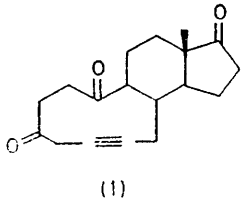
Summary The Michael addition of nucleophiles to the enzyme inhibitor 5,10-seco-oestra-4,5-diene-3,10,17-trione (**2**) gives 5,10-seco-oestr-5-ene derivatives (**3**) or A-homo-B-noroestr-4-ene derivatives (**4**) depending on the nucleophile.

THERE has been considerable interest recently[1–3] in selective irreversible enzyme inhibition by 'suicide substrates.' Many of these compounds contain an acetylenic function which is converted, by the action of the target enzyme, into an electrophilic conjugated allenic grouping. The subsequent irreversible inactivation of the target enzyme is ascribed to Michael addition of an active-site nucleophilic amino-acid side-chain to the conjugated allene.

The identification of the amino-acid residue(s) covalently bound to a given inhibitor of this type is an important but difficult task. Non-enzymatic model studies of such Michael additions, and information about the spectroscopic properties and chemical stability of the resulting adducts would clearly be valuable. Only in one previous case has the postulated conjugated allene been synthesized and subjected to chemical study. Morisaki and Bloch described[4] the Michael addition of histidine and several histidine derivatives to a conjugated allenic thio-ester.



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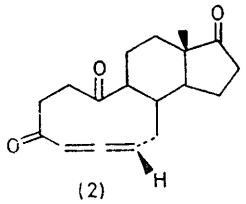

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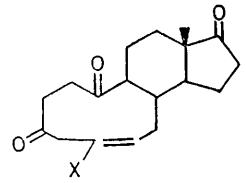

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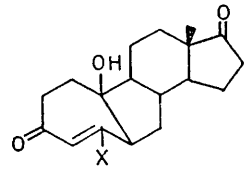

(4)

Our recent work has shown[5,6] that acetylenic 5,10-secosteroids (*e.g.* **1**) are suicide substrates for the enzyme Δ^5-3-ketosteroid isomerase. The derived allenic ketones (*e.g.* **2**) which are generated by, and which irreversibly inactivate, the enzyme have also been prepared non-enzymatically.[6] Preliminary results on the reaction of the allenic ketone (**2**) with water, alcohols, a carboxylic acid, amines, and a thiol are now described. These model nucleophiles contain functional groups representative of nucleophilic side chains of protein amino-acids.

TABLE. Adducts[a] obtained by the action of XH on compound (**2**).

X	Adduct	Conditions[b]	% Yield[c]	M.p./°C
Imidazol-1-yl	(**3a**)	EtOAc; 25 °C	56	187—189
PhS	(**3b**) (*Z*)	MeCN; 25 °C	32[d]	163—164
	(**3c**) (*E*)		25[d]	201—203
PhO	(**3d**)	MeCN–EtN_3; 25 °C	82	111—113
AcO	(**3e**)	MeCN–EtN_3; 25 °C	70	126—127
Pyrrolidin-1-yl	(**4a**)	MeCN; 25 °C	60	156—159
MeO	(**4b**)	MeOH; 65 °C	24[e]	e
HO	(**4c**)[f]	Dioxan–H_2O; 65 °C	48	194—196

[a] Satisfactory microanalytical data were obtained for adducts (**3a**)—(**3e**), (**4a**), and (**4c**). Precise mass measurements on (**4b**) gave *m/e* 318·1805; calc. 318·1831. [b] Unless otherwise specified, reactions were carried out using 2 mol. equiv. of nucleophile per mol. equiv. of allenic ketone (**2**). In some cases, as noted, triethylamine (2 mol. equiv.) was also added. In the cases of adducts (**3a**), (**4b**), and (**4c**), the reactions were carried out respectively in ethyl acetate, in neat methanol, and in 50% aqueous dioxan. The progress of a given reaction was followed by n.m.r. spectroscopy, t.l.c., or h.p.l.c., or a combination of these methods. [c] Yields refer to isolated and purified products. [d] The (*Z*)- and (*E*)-isomers, (**3b**) and (**3c**) respectively, were separated by fractional crystallization from methanol. The crude product comprised (n.m.r.) a 3:2 mixture of (**3b**) and (**3c**). [e] Adduct (**4b**) was isolated by h.p.l.c., and represented the most abundant product. Although homogeneous by chromatographic criteria, (**4b**) could not be induced to crystallize. [f] Adduct (**4c**) was isolated in the β-diketone form.

The major adducts formed in these reactions are shown in the Table. In most cases, the adducts had the unconjugated system (**3**). The geometries of the double bond in the benzenethiol adducts (**3b**) and (**3c**) were established by Raney nickel reduction to the corresponding olefins. The (*E*)- or (*Z*)-configurations of the other adducts of this type have not been assigned rigorously.

On the other hand, addition of water, methanol, or pyrrolidine to (**2**) gave adducts of type (**4**), in which Michael addition was accompanied by ring-closure. *X*-Ray crystallographic studies[7] have established the β-configuration for OH and H at the A/B ring junction of the pyrrolidine adduct (**4a**) [λ_{max} (MeCN) 307 nm; ϵ 26,200]. The structures of the analogous methanol adduct (**4b**) and of the water adduct (**4c**; isolated in the β-diketone form) were assigned on the basis of spectroscopic data, and the A/B ring junction stereochemistry has not yet been established in these cases. The methanol adduct (**4b**) had λ_{max} (MeCN) 250 nm (ϵ12,000). The water adduct (**4c**) was isolated in the β-diketone form and showed in methanolic NaOH the expected u.v. absorption (λ_{max} 294 nm, ϵ 22,900) for the enolate anion of a β-diketone. Acidification of the solution shifted the absorption maximum to 260 nm as expected.

These investigations have provided results which may be generally useful for studies of enzyme-bound adducts derived from conjugated allenes. Studies of the stability towards acid and base of the adducts made in this work, and on the interconversion of conjugated, unconjugated, cyclized, and uncyclized adducts are in progress. These experiments are intended to distinguish between direct formation of adducts of type (**4**) and the initial formation of type (**3**) adducts followed by cyclization.

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