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3β-ACETOXYNORERYTHROSUAMINE, A HIGHLY CYTOTOXIC ALKALOID FROM *ERYTHROPHLEUM CHLOROSTACHYS*

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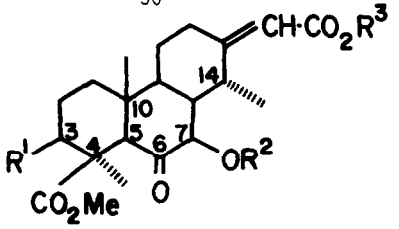
We have recently isolated[1] four natural 3β-acetates of diterpenoid alkaloids from *E. chlorostachys* bark and find them more cytotoxic against KB cell culture[2] than the corresponding 3β-hydroxy alkoids which occur with them. The most toxic of these, which we consider to be 3β-acetoxynorerythrosuamine (1), is a thousand times more active than the parent alcohol (2) and has an ED_{50} value[2] of 0.0003 μg/ml.



	R^1	R^2	R^3
(1)	AcO	H	CH_2CH_2NHMe
(2)	HO	H	CH_2CH_2NHMe
(3)	AcO	Ac	CH_2CH_2NAcMe
(4)	HO	Ac	CH_2CH_2NAcMe
(5)	H	H	$CH_2CH_2NMe_2$
(6)	H	Ac	H

3β-Acetoxynorerythrosuamine has now been purified to give a stable crystalline hydrochloride, m.p. 173-5°: it has already been shown[1] that the new 3β-hydroxy alkaloid and the acetate have the same skeleton and are hydrolysed to 2-methylaminoethanol and a diterpene acid of the C4-CO_2 Me series as evinced by the usual p.m.r. signal ($CDCl_3$) near δ3.75 in spectra of its derivatives. By studying the change in chemical shift of C-methyl signals resulting from acetylation of a hydroxy group in a known orientation we were able to deduce[1] that the hydroxy and acetoxy alkoids are 3β-derivatives.

Acetylation of either base gives the same N,O,O-triacetyl derivative (3) indicating the presence of an additional hydroxy group and a comparison of the C-methyl resonances of thi compound with those of the natural acetate shows changes of shifts similar to those shown by norcassamidine and its N,O-diacetyl derivative, establishing that the second hydroxy group is 7β as in the latter compounds. Mild acetylation of 3β-hydroxynorerythrosuamine for 15 minutes at room temperature with Ac_2O/pyridine gave a crystalline N,7βO-diacetyl derivative (4), m.p. 201°, which again showed shift changes of the same magnitude. These figures are summarised in the Table of p.m.r. date below.

Part of the original p.m.r. evidence[3] for the α-ketol system of erythrosuamine (5) was provided by the H7α signal which has only one coupling, $J_{7,8}$, in place of the usual sextet from $J_{6a,7}$, $J_{6e,7}$ and $J_{7,8}$ and which occurs at lower field than usual as a result of deshielding by the C6 carbonyl group. In erythrosuamine the H7α signal is a doublet at δ3.93 (J 8 Hz), in 3β-hydroxynorerythrosuamine at δ3.94 (J 9 Hz), and in 3β-acetoxynorerythrosuamine at δ 3.94 (J 10 Hz). It shows a typical acylation shift to δ 5.06 (J 11 Hz) in the N,7βO-

COMPARISON OF CHEMICAL SHIFTS OF C-METHYL SIGNALS

Compound	C4-Me	C14-Me	C10-Me
N,O,O,-Triacetyl derivative (3)	1.20	1.14	1.03
3β-Acetate (1)	1.22	1.23	1.01
Shift Difference	-0 .02	-0.09	0.02
N,7βO-Diacetyl derivative (4)	1.35	1.12	0.98
3β-Hydroxynorerythrosuamine (2)	1.37	1.21	0.93
Shift Difference	-0.02	-0.09	0.05
N,7βO-Diacetylnorcassamidine	1.19	1.01	0.65
Norcassamidine	1.20	1.08	0.63
Shift Difference	-0.01	-0.07	0.02

diacetyl derivative and to δ 5.07 (J 11 Hz) in the N,O,O-triacetyl derivative which can be compared with δ 5.19 (J 10 Hz) for the acetate of erythrosuamic acid (6).

The second characteristic feature seen in the spectra[3] of erythrosuamine derivatives is a singlet arising from H5α at δ 2.28 in those derivatives where it is not obscured by signals from the ester side chain. The equivalent singlet occurs at δ 2.33 to 2.35 in 3β-hydroxynorerythrosuamine, the corresponding amide, methyl 3β-hydroxyerythrosuamate, and in the N-acetyl and N,7βO-diacetyl derivatives of 3β-hydroxynorerythrosuamine. The downfield shift of c. 0.05 p.p.m. must be associated with the 3β-hydroxy group, for a further deshielding occurs in the 3β-acetoxy compounds where the singlet is at δ 2.46.

Oxidation of erythrosuamine and related compounds gives an α-diketone. The methyl ester of the 6,7-dioxo acid common to these oxidation products has a diosphenol chromophore with a pH-sensitive u.v. absorption at λ 283 nm (ε 4900) which moves to 337 nm (ε 2400) in alkali[4]. Methyl 3β-hydroxyerythrosuamate behaves similarly in that it is oxidized with the complex from CrO_3-pyridine in methylene chloride to give a triketone as sole product, characterized by its C-methyl signals in the n.m.r. spectrum at δ 1.13, 1.20 (d, J 7 Hz) and 1.35. This compound shows the expected u.v. absorption of an α,β -unsaturated ester at λ_{max} (EtOH) 223 nm (ε 10900) and a second band at 279 nm (ε 1490) which shifts in alkali to 333 nm (ε 1450) as expected.

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