Antitumour Alkaloids of the Vinblastine-type: Air Oxidation of Anhydrovinblastine

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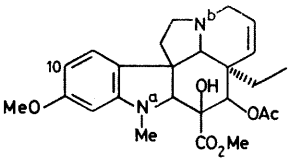
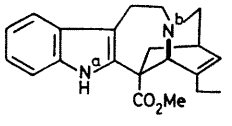
Summary A solution of anhydrovinblastine (**3**) in acetonitrile led, through a spontaneous oxidation–reduction process, not only to leurosine (**4**) and catharine (**9**) but also to other main antitumour alkaloids of *Catharanthus spp.*: vinblastine (**5**), leurosidine (**6**), and their deoxy derivatives (**7**) and (**8**).

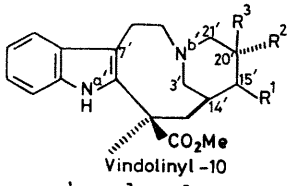
ANHYDROVINBLASTINE (**3**), which is obtained by coupling vindoline (**1**) and the N^b-oxide of catharanthine (**2**) using the modified Polonovski reaction,[1] is rather unstable. We previously suggested[2] that this instability could well explain the absence of this compound amongst the numerous dimeric indole alkaloids isolated from various *Catharanthus spp.* Of course, anhydrovinblastine could be an obvious precursor of most, if not all, dimeric alkaloids of the vinblastine group (**4**)—(**10**).



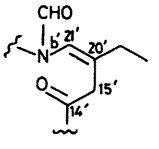
(1)



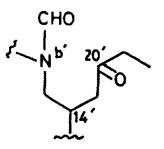
(2)



Vindolinyl-10

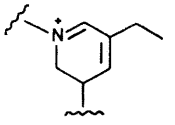


(9)
Catharine



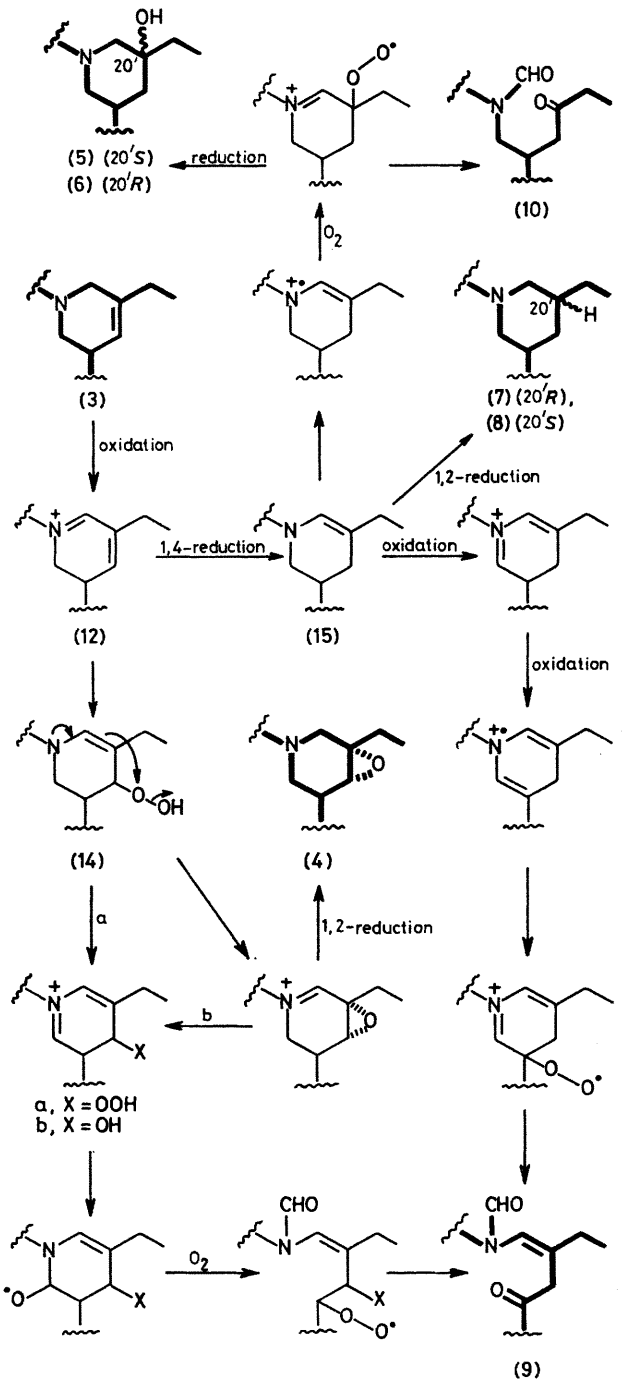
(10)
Catharinine

	R^1	R^2	R^3
(3)	$\Delta^{15'(20')}$		Et
(4)	-O-		Et
(5)	H	Et	OH
(6)	H	OH	Et
(7)	H	Et	H
(8)	H	H	Et
(11)	$\Delta^{15'(20')}$		Et; $N^{b'}$oxide
(13)	CN	Et	; $\Delta^{(20')}$
(15)	H	Et	; $\Delta^{(20')}$



(12)

This has been verified by the recent findings of Guéritte and Scott[3] who demonstrated that anhydrovinblastine (**3**), formed from both (**1**) and (**2**), can indeed be isolated from *Catharanthus roseus* when the extraction procedure is carried out quickly. Furthermore, as we have also postulated,[2] other recent results[4,5] indicate that the structural modifications of the piperidine group of the indolic part of the vinblastine alkaloids (*e.g.* **4**—**10**) take place most probably in the later stages of their biosynthesis.



We have also demonstrated that enzymic systems are not necessary for the transformation of (**3**) into leurosine (**4**) and catharine (**9**)[2] which are formed in significant

yields by simple agitation of a solution of (**3**) in an organic solvent at 25—26 °C (Table, expts. 5 or 8). These *in vitro* experiments were carried out in order to identify the origin (air, oxygen, or water) of the oxygen atoms of the epoxide and $N^{b'}$-formyl groups of, respectively, (**4**) and (**9**), and to study the mechanism of the transformations which lead to these compounds. The main results (Table) allow the following conclusions to be drawn.

TABLE

Expt.	Substrate	Solvent	Conditions:[a] temp.; time; concentration	Compounds obtained (% isolated)
1	(**3**)	Me_2CO	room temp.; 48 h; 15 mM[b]	(**3**) (17), (**4**) (33), (**9**) (7)
2	(**11**)	Me_2CO	room temp.; 48 h; 15 mM[b]	(**11**) (100)
3	(**3**)	MeCN	room temp.; 18 h; 4 mM	(**4**) (23), (**9**) (17)
4	(**3**)	MeCN	room temp.; 18 h; 4 mM[c]	(**3**) (17), (**4**) (21), (**9**) (9)
5	(**3**)	MeCN	12 °C; 19 h,[d] then 25 °C; 24 h; 3·9 mM	(**4**) (22), (**9**) (18), (**5**), (**6**), (**7**), (**8**)[j]
6	(**3**)	MeCN	" " " [e]	(**4**) (24), (**9**) (14), (**5**), (**6**), (**7**), (**8**)[j]
7	(**3**)	MeCN	" " " [f]	(**3**) (19), (**4**) (18), (**9**) (10), (**5**), (**6**), (**7**), (**8**)[j]
8	(**3**)	MeCN	26 °C; 48 h; 5·9 mM	(**4**) (44), (**9**) (12), (**5**) (1), (**6**) (1·2) (**7**) (1·2), (**8**) (1), (**10**) (traces)
9	(**4**)	MeCN	25 °C; 70 h; 3·7 mM	(**4**) (*ca.* 100)
10	(**3**)	MeCN	26 °C; 45 h; 8 mM[g]	(**4**) (34·4), (**9**) (16), (**5**), (**6**), (**7**), (**8**), (**10**)[j]
11	(**3**)	MeCN	26 °C; 46 h; 8 mM[h]	(**4**) (37), (**9**) (17), (**5**), (**6**), (**7**), (**8**), (**10**)[j]
12	(**3**)	MeCN	26 °C; 48 h; 6·6 mM[i]	(**3**) (32), (**13**) (15)

[a] With stirring in air; room temp. = *ca.* 18 °C. [b] In the presence of alumina [weight of (**3**) × 5]. [c] Moisture avoided. [d] Almost no change (t.l.c.). [e] In the dark; bis-(3-t-butyl-4-hydroxy-5-methylphenyl) sulphide added [0·1 mol/mol of (**3**)]. [f] Ethylenediaminetetra-acetic acid [5 mol/mol of (**3**)] added. [g] Addition of H_2O to dry solvent (0·5% v/v). [h] Addition of $H_2{}^{18}O$ to dry solvent (0·5% v/v). [i] KCN [6 moles/mol of (**3**)] and 18-crown-6 [1·1 mol/mol of (**3**)] added. [j] Yields of compounds (**5**)—(**8**) were of the order 1—2%. These yields were estimated and not carefully determined.

The decisive role of the lone-pair of electrons of $N^{b'}$ is proved by the inertness of anhydrovinblastine $N^{b'}$-oxide (**11**) towards air oxidation. The behaviour of anhydrovinblastine (**3**) is very sensitive to temperature but is not significantly modified by adding a radical inhibitor[6] to the reaction medium or by carrying out the reaction in the dark. These observations disprove a radical chain process. In contrast to what has been observed using t-butyl hydroperoxide,[7] under conditions which are known to transform anhydrovinblastine (**3**) into catharine (12—18% yield), leurosine (**4**) does not yield catharine (**9**). This implies that catharine (**9**) is formed from anhydrovinblastine (**3**) in a way parallel to that leading to leurosine (**4**), or it can be formed from a common 'pre-leurosine' intermediate (Scheme). The presence of traces of water seem to favour the transformation of (**3**) (compare expts. 3 and 4). However, there is no ^{18}O incorporation in the isolated compounds (**4**), (**5**), (**6**) and (**9**) when $H_2{}^{18}O$ is added to the reaction medium (expts. 10 and 11). In the presence of potassium cyanide, the major products which result arise from addition of cyanide ion on the conjugated immonium salt (**12**); one of these products, 15-cyano-$\Delta^{20'}$-deoxyleurosidine (**13**) has been obtained previously.[8,9] Other products, vinblastine (**5**), leurosidine (**6**), deoxyvinblastine (**7**), and deoxyleurosidine (**8**) are formed using 'standard' reaction conditions, the latter two being derived from (**3**) by reduction (expt. 8).†‡

Successive oxidation–reduction steps can account for all these results. Although some details are still lacking, the formation of almost all the alkaloids of the vinblastine group from anhydrovinblastine (**3**) can now be rationalized (Scheme).

The conjugated immonium compound (**12**) formed by air oxidation of (**3**) would lead, through 1,4-addition of hydroperoxide, to (**14**), the precursor of leurosine (**4**) and catharine (**9**); 1,4-reduction of the same immonium compound would give the enamine (**15**), the precursor[8,10] of vinblastine (**5**), leurosidine (**6**), and their saturated derivatives (**7**) and (**8**).

In this Scheme, the oxidant is probably oxygen, whilst the reduction must be effected by hydride or hydrogen atom transfer from unoxidised alkaloid.

It is particularly striking that the composition of the mixture of compounds obtained after expt. 8 appears to be very similar to the relative abundance of the dimeric alkaloids isolated from various *Catharanthus* species. In *C. ovalis* Mgf.,[11] leurosine (**4**) and catharine (**9**) predominate over other alkaloids such as (**5**), (**6**), (**7**), or (**8**). However, vincristine (**5** with an N^{a}-formyl instead of N^{a}-methyl group) has not yet been found among the *in vitro* oxidation products of anhydrovinblastine (**3**). All the above results again raise the question of whether the 'down-stream'

† These compounds have been identified by comparison with authentic samples {t.l.c., i.r., u.v., c.d., mass, and ^1H n.m.r. spectra; $[\alpha]_D$ for (**6**) and (**8**)}.

‡ Among additional products which have not been fully characterized, one exhibits an indolenine–indoline u.v. spectrum. It results most probably from a C-7′→C-3′ cyclization of the indole ring on to an immonium at C-3′ (*cf.* 'spontaneous' oxidation of 16*S* methoxycarbonyl-cleavamine in pseudotabersonine: D. Rao *et al.*, unpublished results).

oxidation products of anhydrovinblastine (**3**) are partly or completely 'natural artefacts.'

We thank Professors D. H. R. Barton and E. Wenkert for helpful discussions, Drs. R. Z. Andriamialisoa and S. Kan for ^{1}H n.m.r. spectra at 240 MHz[12] or 400 MHz,[13] and Eli Lilly Laboratories for reference samples.

(*Received, 16th March* 1979; *Com.* 269.)

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