Tetrahedron. Vol 27. pp. 5217 to 5228. Pergamon Press 1971 Printed in Great Britain

ON THE REARRANGEMENT OF CATHARANTHINE, STEMMADENINE AND TABERSONINE IN ACETIC ACID

R. T. BROWN, J. S. HILL, G. F. SMITH and K. S. J. STAPLEFORD
Department of Chemistry, University of Manchester, M13 9PL

(*Received in the UK* 27 *May* 1971; *Accepted for publication* 24 *June* 1971)

Abstract – Experimental details of the reaction of tabersonine (VII) and stemmadenine (XII) in refluxing acetic acid are presented: the main product in the latter reaction is O-acetylstemmadenine (XIII).

The formation of partially racemic pseudocatharanthine (III) both in the treatment of catharanthine (I) with hot acetic acid and in Kutney's cyclising oxidation of 16S-carbomethoxycleavamine (Vb) is discussed.

IN A preliminary communication[1] we discussed in some detail our failure to reproduce the results obtained by Scott and Qureshi on the rearrangement of tabersonine (VII) and stemmadenine (XII) in acetic acid.[2, 3] The main purpose of this paper is to present the experimental details of that part of the work which was carried out in Manchester. Since our interpretation of our observations has not changed. we would refer the Reader to our preliminary paper for its discussion.

The formation of partially racemic pseudocatharanthine

One aspect which we wish to discuss further concerns the formation of partially racemic pseudocatharanthine (III) from (−)-catharanthine (I) and also from 16*S*-carbomethocycleavamine (Vb) (from the latter by oxidative cyclisation[5]).

Rearrangement of catharanthine in refluxing glacial acetic acid. originally reported by Gorman *et al.*.[4] produces pseudocatharanthine whose specific rotation is very low: in four separate experiments running for different lengths of time the $[\alpha]_D$ was −47°. −55°. −60° and −58° (in EtOH). The observed specific rotation of the isolated pseudocatharanthine furthermore did not change on prolonged refluxing in acetic acid. and was already reached after a reaction time of only two hours. This low specific rotation is to be compared with. say. that of the closely related pseudovincadifformine (IVB. 15.20*S*-dihydropseudocatharanthine) which is $[\alpha]_D^{26}$ −503° (EtOH):[5] indeed all alkaloids with the anilinoacrylic ester chromophore. when optically active. have specific rotations in the range 450–700°.

The specific rotation of about −60° observed in pseudocatharanthine is not likely to be due to contamination of fully racemic pseudocatharanthine by about 10% of a different and optically pure base with the same UV and mass spectra. for the 100 hz NMR spectrum shows no signals other than those expected (Fig 1). the compound runs as a single spot in three TLC systems. and catalytic hydrogenation gives one dihydroderivative also homogeneous by TLC (*cf* the stereospecific hydrogenation of 15.20-dehydropseudovincadifformine to a single dihydroderivative[5]). We believe that the above data indicate that the rearrangement of catharanthine (I) to pseudocatharanthine (partially racemic III) proceeds with about 90% racemisation.

The unchanging specific rotation of the pseudocatharanthine on further heating in acetic acid indicates that under the reaction conditions it is optically stable. which

must mean that the observed 90% racemisation is occurring before or in the course of the rearrangement and in a reaction which is not reversibly linked with pseudocatharanthine at any significant rate.

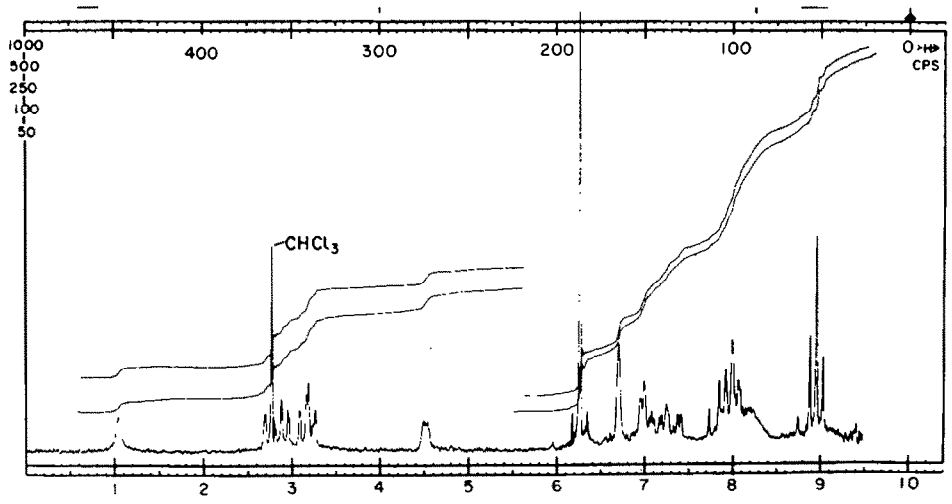


FIG. 1. N.m.r. spectrum of pseudocatharanthine (III).

It is of interest to note at this point that 16*S*-carbomethoxycleavamine[6] (VB)* has been oxidised by Kutney *et al.*[5] to what these Authors call 15.20-dehydropseudovincadifformine (III) which is structurally identical with pseudocatharanthine. The low specific rotation. $[\alpha]_D$ −174°.(EtOH). of the 15,20-dehydropseudovincadifformine. suggests that it. too. is partially racemic. for as already stated above. alkaloids with an anilino acrylic ester chromophore have specific rotations in the range 450 to 700°. Catalytic hydrogenation of the 15.20-dehydropseudovincadifformine gave a dihydro-derivative with $[\alpha]_D$ −172° (EtOH) apparently identical in its spectral and TLC behaviour with the known 20*S*-pseudovincadifformine of $[\alpha]_D$ −503°. previously obtained by Kutney *et al.*[5] by oxidative cyclisation of 16*S*.20*S*-carbomethoxy-dihydrocleavamine (VIC).

The optical purity of the 16*S*-carbomethoxycleavamine (VB) was checked by catalytic hydrogenation to 16*S*.20*S*-carbomethoxydihydrocleavamine (VIc) which had m.p. 149° and $[\alpha]_D$ −65° ($CHCl_3$) corresponding to that reported for the optically pure (VIc) obtained by the zinc and acetic acid reduction of catharanthine.[6]

It would therefore seem that partial racemisation. to the extent of about 65%. is occurring in the oxidative cyclisation of 16*S*-carbomethoxycleavamine (VB). This is to be contrasted with the retention of optical purity in the corresponding oxidative cyclisation of 16*S*.20*S*-dihydrocarbomethoxycleavamine (VIC).[5]

The racemisation process then seems to need the presence of the 15.20-double bond. The simplest rationalisation postulates deprotonation of C14 in the cation (XIVA)

* We have used the biogenetic numbering system for monoterpenoid indole alkaloids,[7] and *R*, *S* nomenclature for denoting configuration. For comparison with the series of paper by Kutney *et al.*, 20 = 4, 16 = 18, R = α and S = β. It should be noted that in some cases partial racemisation may have occurred.

as the potentially racemising step: the resulting intermediate XVA may then either re-protonate at C14 from the same side to return to XIVA or, after a conformational change of the medium ring to XVB undergo C14 protonation from the other side to yield the diastereoisomeric XIVB. The partial nature of the racemisation observed can then be seen as the consequence either of the relative slowness of the conformational change XVA ⇌ XVB or of a rate difference between the ring closure of diastereoisomer XIVA to 14*S*-pseudocatharanthine (III) and the ring closure of diastereoisomer XIVB to the enantiomeric 14*R*-pseudocatharanthine (enantiomer of III).

The question of why partial racemisation is not observed in the oxidative cyclisation of 16*S*,20*S*-carbomethoxydihydrocleavamine (VIc) is difficult to answer: the two main possibilities are (a) that deprotonation of C14 is too slow to compete with C3–C7 bond formation or (b) that the required conformational changes corresponding to XVA → XVB is likewise too slow. A careful study of models does not throw much light on this problem.

The close similarity of the partial racemisations in the oxidation of VB and in the rearrangement of catharanthine (I) to pseudocatharanthine is quite striking, and it is tempting to suggest that the latter rearrangement also involves a 9-membered ring conformational change as the basis of the partial racemisation.

That the catharanthine → pseudocatharanthine rearrangement does not involve 100% racemisation would seem to rule out an achiral dehydrosecodine as an intermediate if one assumes only one rearrangement pathway to be operating. One could however argue that the 10% retention is due to the concurrent operation of a different mechanism, not involving the dehydrosecodines, in which 100% retention of chirality occurs.

Further work on this interesting question is called for.

Analysis of the total reaction mixture obtained by heating catharanthine in acetic acid

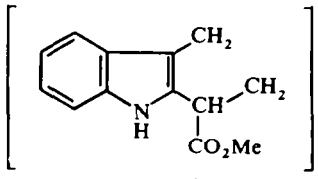
The total reaction mixture obtained by refluxing catharanthine in acetic acid for 2 hr was carefully analysed by preparative TLC which led to the separation of the following compounds (percentages refer to yields estimated UV spectrally): pseudocatharanthine (partially racemic, 30%), coronaridine (IIA, 10%), 20-epipseudovincadifformine (IVB, 5%), pseudovincadifformine (IVA, 5%), 16*S*, 20*R*-carbomethoxydihydrocleavamine (VIB, 3%), 16*R*.20*R*-carbomethoxydihydrocleavamine (VIA, 5%), 16*S*-carbomethoxycleavamine (VB, 2%), and a compound of unknown structure, $C_{21}H_{24}N_2O_4$, which corresponds to the addition of 2 oxygens to the molecular formula of catharanthine (5%). Unchanged catharanthine was not recovered in this reaction, and a run on a larger scale that there was considerably less than 1% remaining after 12 hours. The identifications were carried out by mass spectra and TLC*.

In order to elucidate the structures of some of the products isolated in the acetic acid reaction, we undertook the study of the zinc and acetic reduction of catharanthine and had made considerable progress when Kutney's paper on the same topic appeared.[6] The main difference between the two studies is that whereas the Canadian group report the isolation of one carbomethoxycleavamine, that is the 16*S*-isomer (VB) (from borohydride–acetic acid reduction of catharanthine), we have managed to isolate both the 16*S*- and 16*R*-isomers. The structure assignment for the latter (VA) is made on the basis of mass and UV spectra only because of the small quantities available.

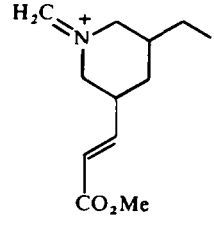
* We wish to thank Professor J. P. Kutney for making this and other comparisons.

so that further confirmation by NMR and conversion into dihydroderivatives remains to be done.

The mass spectrum of the new 16*R*-carbomethoxycleavamine is very interesting and falls nicely into line with those of the 16*S*-epimer and of the four dihydroisomers described by Kutney:[6] the main difference between the mass spectra of the two epimeric carbomethoxy cleavamines lies in the presence in that of the 16*S*-epimer of a strong peak at *m/e* 215 (90–100% of base peak) probably corresponding to XVI, which is absent from that of the 16*R*-epimer. An *m/e* 215 peak is present in the spectra of 16*S*, 20*S*- and 16*S*,20*R*-carbomethoxydihydrocleavamine but absent from the spectra of the 16*R*.20*S*- and 16*R*.20*R*-isomers. So it would seem that the production of the fragment is closely linked with the stereochemistry of the carbomethoxy group, and occurs only with the 16*S* configuration. Given the complex conformational movements of the 9-membered ring in these compounds, a rationalisation of this observation is not possible. Also of interest is the observation that an important fragment at *m/e* 210 is common to the four carbomethoxydihydrocleavamines, but is not observed in either of the C16-epimeric carbomethoxycleavamines: this fragment must be derived from the aliphatic moiety of the molecules, and corresponds to XVII.



m/e 215 XVI



m/e 210 XVII

The C14–C17 bond in the carbomethoxycleavamines is allylic to the C15-C20 double bond, and easier C14–C17 fragmentation at an early stage may account for the absence of the *m/e* 210 fragment.

Reaction of stemmadenine (XII) *with acetic acid*

Since our preliminary communication appeared, we have studied further the reaction of stemmadenine in refluxing acetic acid and have found that the only homogeneous product, formed in about 55% yield after 40 hr, is simply O-acetyl stemmadenine XIII, together with about 5% of unchanged stemmadenine. That the product is the O-acetyl derivative was shown by formation of the same product (mass spectra and TLC) by treatment of stemmadenine with acetic anhydride in pyridine at room temperature, and showing that it is basic, thus ruling out a possible but not probable N(b)-acetyl structure formed by Nb-C21 ring cleavage and cyclic ether formation between the CH_2OH and C19 or C21. To make sure that O-acetylation was not the consequence of the presence of a trace of Ac_2O in the AR acetic acid used, we repeated the reaction with acetic acid which had been refluxed and distilled from *p*-aminobenzoic acid: O-acetylstemmadenine was still produced.

Further work on the catharanthine-pseudocatharanthine rearrangement is in hand.

EXPERIMENTAL

The AcOH used was A.R. grade, redistilled prior to use. All reaction solns were degassed and thereafter kept under O_2-free N_2. The reaction flasks were immersed in oil baths up to the level of the soln inside.

TLC was usually performed on pre-coated silica F_{254} or alumina F_{254} (0·25 mm thick) and preparative (2 mm thick) plates supplied by Merck. $AgNO_3$ impregnated plates were made with silica G(35 g), fluorescent indicator (0·35 g) and 3% $AgNO_3$ aq (60 ml) and left to air-dry overnight; immediately before use they were activated by heating at 50° for 1 hr. The systems used were as follows:-

A: silica F/benzene. EtOAc. MeOH (2:2:1); B: silica F/$CHCl_3$. EtOAc (9:1); C: silica F/ether: D: silica G/$AgNO_3$/petrol (40–60°). ether (2:1); E: alumina F/benzene; F: alumina F/benzene. EtOAc (3:1); G: silica F/EtOH, EtOAc (1:9).

Spots were visualised by quenching of fluorescence and by spraying with a sat soln of ceric sulphate in 6N HSO_4. which gave characteristic colours (Table for summary of data).

Mass spectra were run on AEI MS9 and MS12 spectrometers; the intensities of peaks are quoted in terms of % abundance relative to the base peak.

UV spectra were recorded in MeOH soln on a Unicam SP800 spectrometer.

NMR spectra were obtained on a Varian HA100 spectrometer.

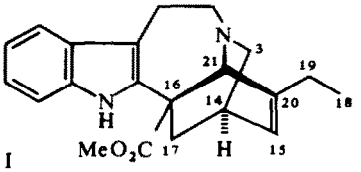
Compound	R_f values in system					Ceric colour
	A	B	C	E	F	
Catharanthine I	0·55	0·15	0·53		0·56	blue
Coronaridine IIA		0·45	0·84	0·33		blue
Dihydrocatharanthine IIB		0·15			0·59	blue-purple
Pseudocatharanthine III	0·63	0·52	0·80			blue
20*R*-Dihydropseudocatharanthine IVA		0·37		0·22		blue
20*S*-Dihydropseudocatharanthine IVB		0·32		0·22		blue
16*R*-Carbomethoxycleavamine VA		0·28	0·75	0·30		purple
16*S*-Carbomethoxycleavamine VB		0·63	0·83	0·30		blue
16*R*.20*R*-Carbomethoxydihydro-cleavamine VIA		0·38		0·41		pink
16*S*.20*R*-Carbomethoxydihydro-cleavamine VIB		0·62	0·83	0·32		pink
16*S*.20*S*-Carbomethoxydihydro-cleavamine VIC		0·60	0·84	0·31		pink-blue
Tabersonine VII	0·63	0·52	0·80			blue
Vincadifformine VIII		0·42				blue
Allocatharanthine IX		0·12	0·36		0·49	blue
Dihydroallocatharanthine X		0·13	0·53		0·56	purple
Acetoxyallocatharanthine XI		0·20	0·53		0·46	orange
Stemmadenine XII	0·15					crimson
O-Acetylstemmadenine XIII	0·23					crimson

TABLE 1.

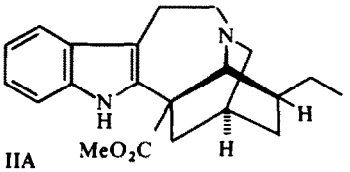
Rearrangement of catharanthine (I) *in acetic acid*

This reaction was carried out several times with varying oil-bath temp (140–180°) for 2–12 hr. with similar results in all cases. The following is typical of the general procedure followed.

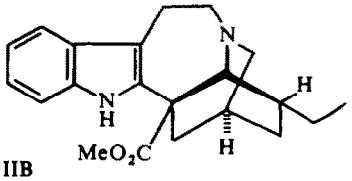
A soln of catharanthine (190 mg) in AcOH (25 ml) was refluxed under O_2-free N_2 in an oil-bath at 140–160° for 2 hr. The solvent was removed under reduced pressure and any residual AcOH removed by repeated evaporation with benzene. The total residue was initially fractionated by TLC on system B using three preparative plates (20 × 20 cm) to give seven fractions:



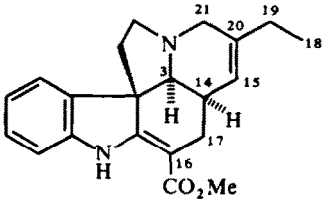
I



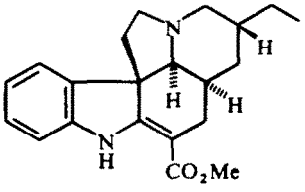
IIA



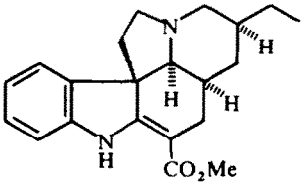
IIB



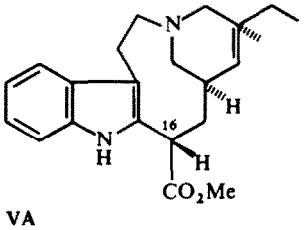
III



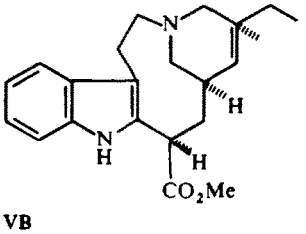
IVA



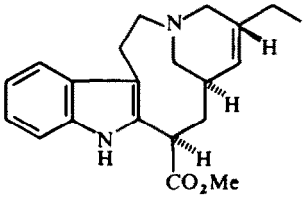
IVB



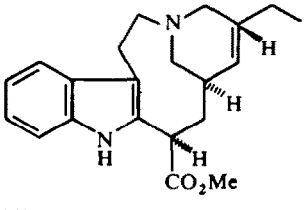
VA



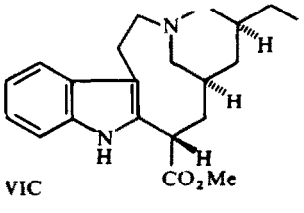
VB



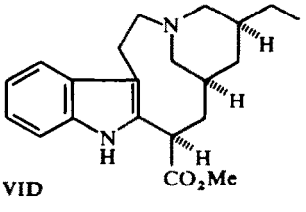
VIA



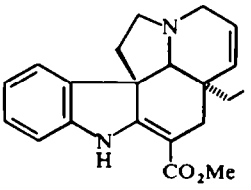
VIB



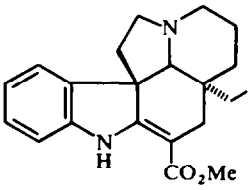
VIC



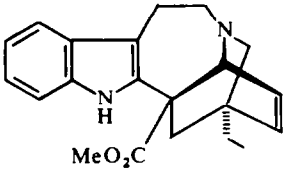
VID



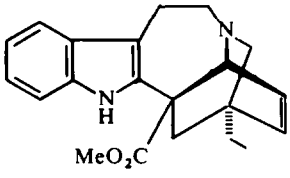
VII



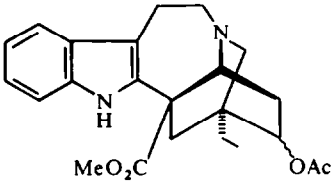
VIII



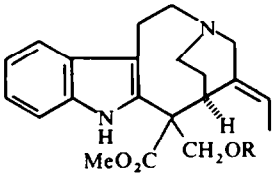
IX



X

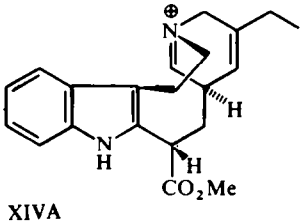


XI

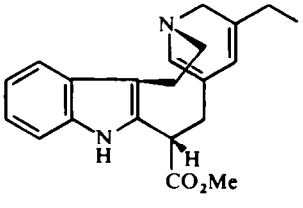


XII: R = H

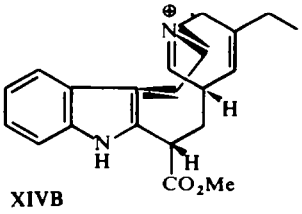
XIII: R = Ac



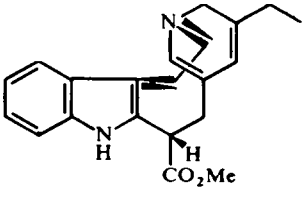
XIVA



XVA



XIVB



XVB

Fraction	R_f	*Wt (mg)*	Ce^{4+} *colour*
1	0·85–0·73	6	mauve
2	0·73–0·62	66	blue/yellow centre
3	0·62–0·52	13	mauve
4	0·52–0·30	21	blue
5	0·30–0·18	10	blue
6	0·18–0·14	8	blue
7	0·14–0·00	13	brown

Fraction 2 had the spectral properties expected of III: λ_{max} (log ε) 325 (4·21), 295 (4·09) nm; τ ($CDCl_3$) 9·0 (—CH_2—CH_3), 8·0 (—CH_2—CH_3), 6·3 (—OCH_3), 4·5 (= C - H), 1·1 (NH); *m/e* 336 (68), 138(100), 122(32), 108(36). It gave a crystalline picrolonate m.p. 210° (dec). The base was optically active. $[\alpha]_D^{25}$ (EtOH) varied slightly for different batches: −55·2° and −58·5° (12 hr reflux), −46·6° and −60°(2 hr reflux).

Fraction 3 gave the following spectral data: λ_{max} 228, 286, 293 nm; τ ($CDCl_3$) 9·10 (—CH_2—CH_3), 6·32 (—OCH_3), 2·22 (NH); *m/e* 338 (M^+ 100), 136(50), 124(30). It was identified as coronaridine (IIA) by TLC, mass and NMR spectral comparison with an authentic sample prepared by epimerising dihydrocatharanthine according to the published procedure.[4]

Fraction 6 was homogeneous by TLC and gave the following data: λ_{max} 226, 285, 292 nm; *m/e* 368(72), 228(36), 214(46), 201(39), 169(100), 156(55), 154(65). The structure has not yet been determined but it corresponds to catharanthine plus two oxygens: M^+ 368·1739; $C_{21}H_{24}N_2O_4$ requires 368·1736.

Fraction 1 was inhomogeneous and hence was run again on a preparative plate in system B. The major cut (3·4 mg) consisted of two incompletely resolved components which could only be separated using an analytical plate (20 × 20 cm) in system E with multiple development, fractionating and repeating the separation.

The upper component, 1A, (R_f 0·32 in the latter system) gave a pink colour with ceric reagent, had an indolic chromophore and M^+ 340; it was identified as VIB by direct comparison with authentic material (TLC, MS, and IR).*

The lower component, 1B, (R_f 0·30) gave a blue colour with ceric reagent, had an indolic chromophore and M^+ 338; it was identified as VB.

Fraction 4 was also run again on a preparative plate in system E, and after double development separated into two bands. The upper, 4A, (2 mg) was found to be identical with 5A1/5B1 (0·5 mg) and hence was combined with them. It gave a pink colour with ceric reagent, had an indolic chromophore, and M^+ 340; it was identified as VIA.

The lower band, 4B (8 mg) was inhomogeneous; TLC on a preparative plate (20 × 20 cm) in system B gave upper and lower fractions, 4B1 and 4B2 respectively. The former (2·8 mg) was identical with fraction 3, identified as IIA.

The latter fraction (3·6 mg) was combined with fraction 5B2 (2·0 mg) they had identical compositions (see below). By plating twice on six analytical plates (10 × 20 cm) in system B resolution was achieved into an upper component, 4B2a (2·2 mg) and a lower 4B2b (1·6 mg), both of which gave blue spots with ceric reagent.

Fraction 4B2a had λ_{max} 298, 325 nm., and *m/e* 338 (M^+, 22), 124(100) and was identified as 20*R*-dihydropseudocatharanthine (pseudovincadifformine) (IVA) by direct comparison with an authentic sample.

Fraction 4B2b also had a β-anilinoacrylic ester chromophore and similar but not identical IR and mass spectra to the above. It was identical to a 15,20-dihydropseudocatharanthine obtained by hydrogenation of III and hence corresponds to the 20*S* isomer IVB.

Fraction 5 was run again on a preparative plate (20 × 20 cm) in system B, and two continuous major bands were cut out, an upper 5A (1·0 mg) and a lower 5B (3·5 mg). These were run again on analytical plates (20 × 20 cm) on system E, when each gave two bands: 5A1 (0·2 mg) 5A2 (0·12 mg), 5B1 (0·25 mg), and 5B2 (2·0 mg). 5A1 and 5B1 were identical with fraction 4A (16*R*, 20*R*-carbomethoxydihydrocleavamine). 5B2 had the same composition as 4B2 and was combined with it.

Fraction 7 contained polar material and was not examined further.

Estimation of residual catharanthine after acetic acid reaction

Catharanthine (497 mg) in AcOH (10 ml) was refluxed under N_2 for 12 hr at a bath temp of 130–140° and the product isolated as above. Analytical TLC on system B did not reveal any catharanthine, and the

* We wish to thank Professor J. P. Kutney for making this and other comparisons.

predominant UV chromophore was that of a β-anilinoacrylic ester. Part of the reaction product (164 mg) was fractionated by preparative TLC on system B and elution of the region corresponding to catharanthine gave 23·6 mg. (The total recovery of material was 80%, and it had previously been shown that catharanthine could be recovered with 94% efficiency from the system used). The material from the catharanthine region was run on a preparative plate in system G to give four fractions (total recovery 88%) of which one (1·7 mg) was indolic and corresponded to catharanthine. Analytical TLC indicated that it contained three components one of which had the same R_f value, ceric reaction and mass spectrum as catharanthine.

Thus the amount of catharanthine remaining after 12 hr cannot be greater than about 1%, and is probably considerably less.

Zinc-acetic acid reduction of catharanthine

A soln of catharanthine (100 mg) in AcOH (5 ml) was heated with Zn dust (1 g) under reflux and N_2 for 4 hr. The soln was then decanted, evaporated and residual AcOH removed by azeotroping with benzene. TLC on two preparative plates (20 × 20 cm) in system B gave six fractions numbered 1–6 in order of decreasing R_f.

Fraction 1 (9·5 mg) was plated again in system B and multiple development separated two bands—1A (mauve ceric colour) and 1B (pink-blue ceric colour). 1A on running in system E gave two components, 1A1 (pink ceric colour) and 1A2 (pink-blue ceric colour). The former was an indole, M^+340, and was identified as VIB; the latter was an indole, M^+338, identified as VB by direct comparison. 1B was not resolvable by TLC on any of the usual systems, but the mass spectrum showed that the major component had M^+340 and a minor component, M^+338. The UV spectrum showed only an indolic chromophore. Direct TLC comparison indicated the presence of VIC.

Fraction 2 (1 mg) was purified by running again on system B and subsequently shown to be III by direct comparison with an authentic sample.

Fraction 3 (5 mg) after plating in system E afforded an indole, M^+338, which was shown to be IIA by direct comparison.

Fraction 4 (14 mg) was re-plated in system E and the major component (pink ceric colour) isolated. This was an indole, M^+340, whose mass spectrum was similar to that of 1A1; it was identified as VIA by comparison with authentic material.

Fraction 5 (6·5 mg) was run again in system B and partially resolved into two bands 5A (upper) and 5B (lower, purple ceric colour). The former was not homogeneous but plating in system C removed some of component 4 to afford 5A as a pure compound which gave an orange ceric colour. Both 5A and 5B were indoles and showed M^+338. The mass spectrum of 5B closely resembled that of VB but was not identical and hence it probably corresponds to VA. No assignment could be made for 5A.

Fraction 6 (20 mg) contained much polar material and was not investigated further.

Rearrangement of pseudocatharanthine

Rigorously purified III (120 mg) $[\alpha]_D^{25}$-60° (EtOH) was refluxed under N_2 in AcOH (25 ml) for 50 hr. The solvent was then removed, the residue divided into two equal parts and one of these partitioned between chloroform and 1N Na_2CO_3. TLC examination of the chloroform soluble material (57 mg) on system B showed that it consisted mainly of unchanged pseudocatharanthine, together with IIA and IVA and B. A preparative plate afforded three fractions: (1) pseudocatharanthine (24 mg) $[\alpha]_D^{25}$–60° (EtOH), (2) coronaridine and dihydropseudocatharanthines (8·8 mg) and (3) a mixture with R_f 0·05–0·35 (8·7 mg).

Fraction 3 was run again in system F and contained at least ten compounds R_f 0·0–0·60. Two of these R_f 0·59 (blue-purple ceric colour), and R_f 0·56 (blue ceric colour) were separated by preparative TLC. The former was apparently IIB (1·3 mg) and the latter I (0·5 mg) by comparative UV and mass spectra and TLC.

16S,20S-*Carbomethoxydihydrocleavamine.* 16*S*-Carbomethoxycleavamine m.p. 121–3°. $[\alpha]_D^{25}$ + 41° (EtOH) (23 mg) was hydrogenated in EtOAc over Adams' catalyst for 5 hr to give VIC which recrystallised from MeOH as blades, m.p. 149°. $[\alpha]_D^{25}$ −65° ($CHCl_3$).[6]

Dihydropseudocatharanthine. Pseudocatharanthine $[\alpha]_D^{25}$ − 172° (EtOH), prepared from the above 16*S*-carbomethoxycleavamine, was hydrogenated with Pd/C according to the published procedure[5] to give dihydropseudocatharanthine (pseudovincadifformine) $[\alpha]_D^{25}$ − 174° (EtOH).

Rearrangement of tabersonine (VII)

(A) A soln of tabersonine (14 mg) in AcOH (5 ml) was refluxed under N_2 for 18 hr (oil-bath temp 190–5°). The solvent was removed *in vacuo* and the residue partitioned between chloroform and N Na_2CO_3.

Evaporation of the chloroform extract gave a gum (12 mg), which an examination by TLC on system B showed to be a mixture. The major component had R_f 0·52 which corresponded to both tabersonine and III. Preparative TLC on 9 mg of reaction product afforded 4·1 mg of the R_f 5·2 material, which had a β-anilinoacrylic ester chromophore and was not resolved from tabersonine on several TLC systems. In particular, using system D under conditions which were simultaneously demonstrated to separate tabersonine R_f 0·52 from pseudocatharanthine R_f 0·65.* only tabersonine and *no* pseudocatharanthine could be detected.

Furthermore, hydrogenation of the R_f 0·52 material (2·4 mg) in EtOH (5 ml) over Adams' catalyst (5 mg) resulted in uptake of 1·1 equivs of hydrogen. TLC examination of the product on system B showed that it had the same R_f value as dihydrotabersonine (vincadifformine; VIII R_f 0·42, and with ceric reagent gave an identical initial blue colour fading to brownish purple. No starting material R_f 0·52 or IV R_f 0·45 (corresponding to that produced by hydrogenation of pseudocatharanthine) could be detected. The mass spectrum of the product was identical with that of vincadifformine, but showed slight intensity differences from that of dihydropseudocatharanthine when all spectra were run consecutively under conditions as nearly identical as possible: thus for instance vincadifformine had *m/e* 253 > *m/e* 180 whereas the converse applied to dihydropseudocatharanthine.

Therefore it would appear that pseudocatharanthine is *not* present among the rearrangement products of tabersonine.

(B) The reaction of tabersonine (13·6 mg) with AcOH was repeated as above but with an oil-bath temp of 200° and extended reaction time of 50 hr. The chloroform soluble product (11·2 mg) was examined by TLC on several systems and had the same qualitative composition as in A. Preparative TLC on system C of 9 mg of reaction product gave three major fractions. (1) R_f 0·80. (2) R_f 0·53, and (3) R_f 0·36. The zone intermediate between (2) and (3) would have contained any catharanthine, and was also extracted and examined by UV and mass spectra and by TLC. It contained less than 0·1 mg of indolic material, by UV, and no catharanthine could be detected by TLC even on heavy loading (0·1 mg).

Fraction (1) (2·9 mg) was found to be unchanged tabersonine.

Fraction (2) (0·84 mg) gave a blue ceric reaction and had an indolic UV with traces of a β-anilinoacrylic ester chromophore, and M^+ 336. Initially it was shown *not* to be catharanthine, and eventually was shown to be identical with IX (supplied by Professor Poisson) by comparative TLC and mass spectra.

Fraction (3) was run again on system B and resolved into two indolic components: (a) R_f 0·20 (0·51 mg) gave an orange ceric reaction and (b) R_f 0·13 (0·48 mg) gave a purple ceric reaction. Added catharanthine (R_f 0·15, blue ceric reaction) was clearly resolved from both components on multiple development in system B. Fraction (3a) was identified as XI, and (3b) as XII by comparison of their mass spectra with those of authentic samples supplied by Professor Poisson.

The products isolated from tabersonine after 50 hr reflux in acetic acid were:–

Unchanged tabersonine (VII) 26%; allocatharanthine (IX) 9%; dihydroallocatharanthine (X) 5%; acetoallocatharanthine (XI) 4%. Three other reactions were also performed under varying conditions with essentially the same results:

(i) Tabersonine (5·0 mg) in redistilled (but not degassed) AcOH (2 ml) refluxed under N_2 for 16 hr at a bath temp of 130–145°.

(ii) Tabersonine (16·6 mg) in degassed AcOH (2 ml) refluxed under N_2 for 16 hr at a bath temp of 204–6°.

(iii) Tabersonine (4 mg) in degassed AcOH (2 ml) heated in an evacuated sealed tube for 16 hr at a bath temp of 118°.

In no case was any catharanthine or pseudocatharanthine detected.

Standardisation of tritiated acetic acid

A soln of catharanthine (30 mg) in CH_3CO_2T (1 ml approx 200μC) was refluxed under N_2 for (i) 2 hr and (ii) 40 hr with an oil-bath temp of 130–150°. The *same* tritiated AcOH was used for both experiments, and for the rearrangement of XII (see below) which was performed *between* the two catharanthine reactions. After removal of the AcOH by lyophilisation, any labile tritium was exchanged by repeated evaporation of the residue with MeOH. Filtration of a chloroform soln through grade II neutral alumina and evaporation

* System D was the only one which could achieve a separation, and even then the activation of the plates is crucial and a matter of trial and error for each batch. It is essential to avoid both over-activation which causes streaking and loss of resolution, and under-activation which leads to inhomogeneity and subsequent wandering of the spots. We thank Professor Scott for communicating this method to us.

gave a gum (25 mg). In each case, half of this material was fractionated by preparative TLC on system B to give (i) 4 mg and (ii) 1·4 mg of pseudocatharanthine which was diluted with (i) 28 mg and (ii) 8·4 mg of cold material. Addition of an equivalent of picrolonic acid in methanolic soln afforded the picrolonated salt, which on recrystallisation from MeOH gave yellow rods m.p. 210° (dec). Recrystallisation to constant activity gave specific activities of (i) $1{\cdot}30 \times 10^6$ and (ii) $3{\cdot}45 \times 10^6$ dpm/mg, which correspond to activities of (i) $1{\cdot}86 \times 10^7$ and (ii) $6{\cdot}56 \times 10^7$ dpm/mg for the undiluted pseudocatharanthine.

TLC analysis again showed that there was little catharanthine left even after 2 hr.

Attempted rearrangement of stemmadenine (XII) *in acetic acid*

A. *In tritiated acetic acid.* A soln of stemmadenine (8·1 mg) in CH_3CO_2T was refluxed under N_2 for 40 hr with an oil-bath temp of 130–150°. After removal of the AcOH and exchangeable tritium in the usual way, catharanthine (30 mg) and pseudocatharanthine (30 mg) were added, the mixture taken up in chloroform and filtered through neutral alumina (grade II). Evaporation afforded a gum (65 mg) which was fractionated by preparative TLC on system B to give pseudocatharanthine (21 mg) R_f 0·52 and catharanthine (30 mg) R_f 0·15.

The pseudocatharanthine was converted into its picrolonate which was recrystallised to a constant activity of 357 dpm/mg, corresponding to a *total* pseudocatharanthine activity of $1{\cdot}9 \times 10^6$ dpm. Hence the maximum yield of pseudocatharanthine (from 8·1 mg stemmadenine) is approximately

$$\frac{1{\cdot}9 \times 10^6}{6{\cdot}56 \times 8{\cdot}1 \times 10^7} = 0{\cdot}004\%$$

The catharanthine was converted into its hydrochloride which was recrystallised from MeOH–ether until a constant specific activity of 178 dpm/mg was reached. This corresponds to a total catharanthine activity of $5{\cdot}4 \times 10^5$ dpm.

B. *In refluxing acetic acid.* A soln of stemmadenine (4·7 mg) was refluxed under N_2 for 40 hr at an oil-bath temp of 280°. The solvent was removed and the residue (indole chromophore) partitioned between EtOAc and N $NaCO_3$. The aqueous layer contained only a trace of material, λ_{max} 268 nm, and virtually all the indolic material (3·5 mg) was in the organic layer. TLC examination of the EtOAc extract on system A showed two components, R_f 0·15 and 0·23, which both gave crimson ceric reactions. Catharanthine (R_f 0·55), tabersonine (0·63) and pseudocatharanthine (0·63), which all give blue ceric reactions, were not detected even at heavy loading. The products were separated by preparative TLC on system A and the lower R_f material (0·24 mg) identified as unchanged stemmadenine by TLC and mass spectral comparison. The upper material (2·9 mg) had M^+ 396 (23%), *m/e* 337(14) 277(10) and 123(100); its identity with XIII was confirmed by direct TLC and mass spectral comparison with an authentic sample prepared by acetylation of stemmadenine with acetic anhydride-pyridine.

The area between R_f 0·30 and 1·0 of the preparative plate was also eluted and the residue after evaporation examined by TLC, UV and mass spectroscopy, again with failure to detect any catharanthine, pseudocatharanthine or tabersonine.

In view of the formation of the acetate, the AcOH for a subsequent experiment was refluxed with *p*-aminobenzoic acid before distillation to ensure absence of traces of AcO. Repetition of the reaction on stemmadenine (4·7 mg) and taking a sample after 1 hr and working up after 14 hr gave the following results:

Time (hr)	*% Stemmadenine*	*% Acetate*
1	major component	trace
14	15	50
40	5	55

The reaction was performed twice more under varying conditions with similar results:–

(i) Stemmadenine (5·2 mg) in redistilled but not degassed acetic acid (2 ml) refluxed under N_2 for 33 hr at a bath temp of 140°.

(ii) Stemmadenine (9·2 mg) in degassed AcOH (2 ml) refluxed under N_2 for 72 hr at a bath temp of 206–216°.

C. *In a sealed tube.* Stemmadenine (5·6 mg) in AcOH (5 ml) was heated in an evacuated sealed tube immersed in refluxing AcOH (118°) for 34·5 hr. The tube was then opened and the solvent removed under reduced

pressure. The residue had a purely indolic UV spectrum with no absorption in the 320–330 nm region characteristic of αβ-anilinoacrylic ester chromophore. No tabersonine, pseudocatharanthine or catharanthine could be detected by TLC or mass spectral analysis.

We wish to thank Drs. N. Neuss. D. Stauffacher and J. Le Men for gifts of alkaloids and Professors J. P. Kutney and J. Poisson for their collaboration in exchange and identification of samples.

REFERENCES

[1] R. T. Brown. J. S. Hill. G. F. Smith and K. S. J. Stapleford and J. Poisson. M. Muquet. and N. Kunesch. *Chem. Comm.* 1475 (1969)
[2] A. A. Qureshi and A. I. Scott. *Ibid.* 945 (1968)
[3] *Ibid.* 947 (1968)
[4] M. Gorman. N. Neuss. and N. J. Cone. *J. Am. Chem. Soc.* **87**. 93 (1965)
[5] J. P. Kutney. R. T. Brown. E. Piers. and J. R. Hadfield. *Ibid.* **92**. 1708 (1970)
[6] J. P. Kutney. W. J. Cretney. J. R. Hadfield. E. S. Hall. V. R. Nelson. *Ibid* **92**. 1704 (1970)
[7] J. Le Men and W. J. Taylor. *Experientia.* **21**. 508 (1965)