Total synthesis of indole and dihydroindole alkaloids. XII.[1,2] Selective functionalization of various bisindoles. Efficient syntheses of leurosine and related bisindole alkaloid derivatives

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Studies involving selective functionalization of the indole unit in a number of bisindole alkaloids and related synthetic derivatives are described. These investigations include such reagents as *tert*-butyl hydroperoxide, which provides an efficient procedure for epoxidation of olefinic linkages, mercuric acetate, osmium tetroxide, and iodine under basic conditions and oxygenation under acidic conditions. Efficient syntheses of leurosine (**8**) and various novel vinblastine derivatives are developed.

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On décrit des études impliquant la fonctionnalisation sélective d'unités indoliques dans un certain nombre d'alcaloïdes dérivés du bisindole et de dérivés de synthèse qui leur sont reliés. Ces études comprennent des réactifs tels que: l'hydroperoxyde de *tert*-butyle qui permet d'effectuer d'une façon efficace l'époxydation de doubles liaisons, l'acétate mercurique, le tétroxyde d'osmium et l'iode en milieu basique ainsi que l'oxygénation en milieu acide. On a développé des synthèses efficaces de la leurosine (**8**) et de divers dérivés nouveaux de la vinblastine.

[Traduit par le journal]

In a continuing research program directed at the laboratory synthesis of bisindole alkaloids of the vinblastine–vincristine series, we have considered various approaches in the hope of developing efficient procedures for the preparation of these clinically important agents. Our previous investigations (1–3, 5) involved studies in which appropriate indole and dihydroindole units were coupled to provide the desired bisindole systems. In another study we have considered the development of selective reactions which would allow specific functionalization of appropriate bisindole systems already available from previous experiments. The results presented herein illustrate the success of such an approach.

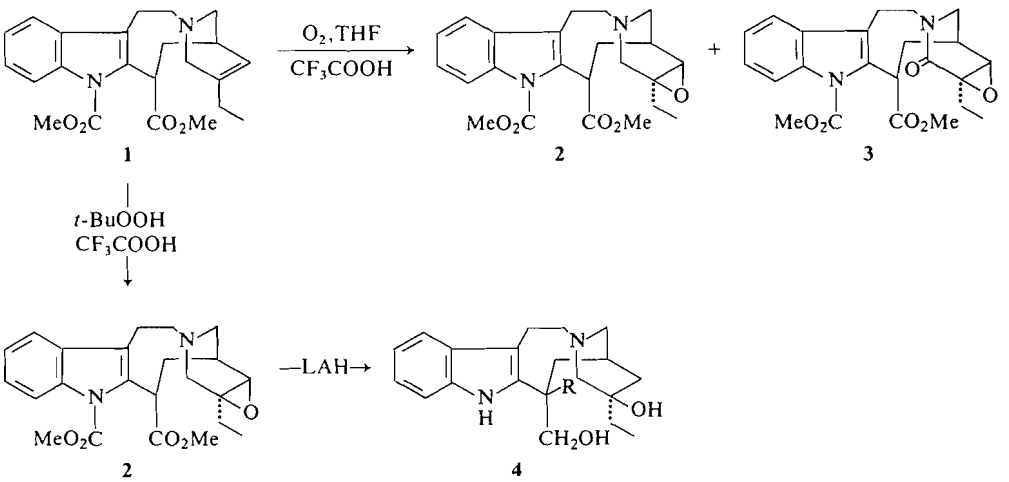
It has already been noted (Part XI, ref. 4) that electrophilic addition to the olefinic linkage of the catharanthine system is complicated by competitive side reactions at the indole or the basic nitrogen site in the molecule. A similar difficulty was observed with the cleavamine system, the indole unit normally present in the bisindole alkaloids. After much frustration with the more common electrophilic reagents generally employed in olefin functionalization, we turned our attention to oxygen and hydroperoxide oxidation of alkenes, a reaction which is not generally utilized by synthetic chemists (6–9).

The intermediate chosen for this study was N_a-carbomethoxy-18β-carbomethoxycleavamine (**1**) since this compound was expected to exhibit properties similar to those of the indole unit present in the bisindole alkaloids. The preparation of this intermediate was accomplished by reacting the readily available 18β-carbomethoxycleavamine (1, 10) with strong base to remove the indolic hydrogen and treating the resultant anion with methyl chloroformate.

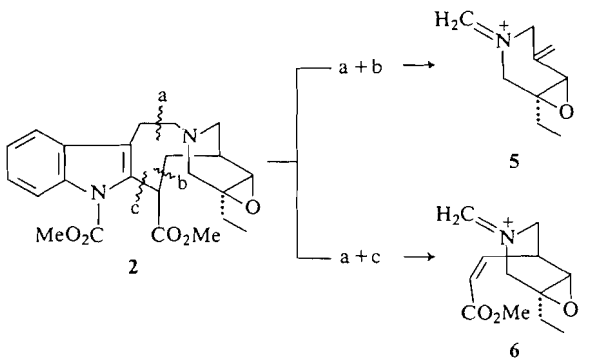
Oxidation of **1** with oxygen in a tetrahydrofuran solution containing trifluoroacetic acid provided two main products, which on the basis of their spectral data, were assigned the structures **2** and **3**. The major component (51% yield) was the lactam epoxide **3** while the desired N_a-carbomethoxy-18β-carbomethoxy - 3β,4β - epoxydihydrocleavamine (**2**) was isolated in only 10–15% yield. Of particular importance in allowing the structural assignment for **2** was the characteristic fragmentation pattern in the mass spectrum. The most significant fragmentation occurs in the manner indicated with cleavage at 'a' and 'b' providing the ion **5** (*m/e* 152, 17%; $C_9H_{14}NO$

[1]For part XI see ref. 5.

[2]For preliminary reports on portions of this work, see ref. 19.



requires 152.107; found: 152.106) and at 'a' and 'c' to provide ion **6** (*m/e* 224, base peak; $C_{12}H_{18}NO_3$ requires 224.129; found: 224.128).



Clearly the data provided thus far did not allow any stereochemical assignment to the epoxide ring as shown in **2** and **3** but further chemical evidence settled this question. Lithium aluminum hydride reduction of **2** in refluxing *N*-methylmorpholine provided the diol **4** (R = H) in 80% yield. The structure of the latter follows directly from the fact that the known triol **4** (R = OH) available from a previous study (11) could be similarly reduced to **4** (R = H). Since the interrelationship of the triol (**4**, R = OH) with isovelbanamine (11) firmly establishes the absolute configuration of the tertiary hydroxy group as shown, it is clear that the epoxide ring in the oxidation products **2** and **3** must be in a β orientation.

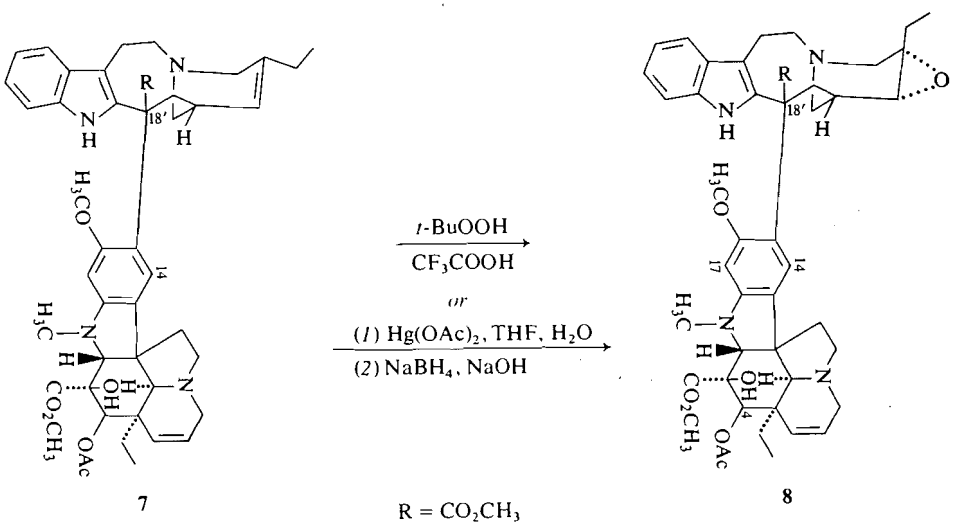
Further studies to improve the yield of the desired epoxide intermediate **2** in the oxygenation reaction met with failure but reaction of **1** with the hydroperoxide in aqueous trifluoroacetic acid provided **2** in 67% yield and thereby suggested an attractive application of this reaction to the bisindole series.

The most attractive intermediate from the bisindole series which appeared suitable for studies with the hydroperoxide procedure was 3′,4′-dehydrovinblastine (**7**), a synthetic vinblastine analogue available from our earlier studies (2, 3) and independent investigations conducted elsewhere (12, 13). This compound was treated with *tert*-butyl hydroperoxide and trifluoroacetic acid in tetrahydrofuran as solvent. The product, obtained in 51% yield, was identified as the bisindole alkaloid leurosine **8** by appropriate comparisons with an authentic sample. As already indicated in the previous publication (Part XI, ref. 5) the stereochemistry of the epoxide ring in this alkaloid is as shown in **8**, thereby establishing attack of the hydroperoxide from the α face of the indole unit in **7**. The epoxidation process was selective in that products resulting from reaction at the olefinic linkage in the vindoline unit of **7** were not observed.

An even more efficient route to leurosine (62%) was realized by treatment of the hydrochloride salt of **7** with mercuric acetate and subsequent quenching with sodium borohydride.

In recent isolation studies it has been demonstrated that vicinal diols are present in some bisindole alkaloids as exemplified by vincadioline (14). Thus it appeared appropriate to evaluate selective diol formation with 3′,4′-dehydrovinblastine in the hope that such studies would allow entry into this series of compounds. For this purpose a detailed investigation with osmium tetroxide was initiated and a summary of the results is presented in Fig. 1.

It was found that the nature and relative yields of the products obtained from osmylation of **7** depended markedly on the reaction conditions employed. Initial investigations with **7** and osmium tetroxide revealed a complex reaction product mixture but results with the *N*-oxide derivative **9** were much more encouraging and further studies were performed.



7 R = CO_2CH_3 8

Reaction of **9** with 1 or less than 1 equiv. of osmium tetroxide provided two main products which from spectral and chemical data could be assigned the structures **10** (R_1 = OH) and **11** (R_2 = O).

The major component (53% yield) was 3′,4′-dehydro-19′-oxovinblastine (**11**, R_2 = O) and represented a product in which normal hydroxylation of the olefinic linkage in the indole unit had not occurred but rather oxidation at the basic nitrogen site to provide the lactam. The expected product resulting from hydroxylation of the 3′,4′-double bond in **9** and tentatively assigned the name 3′-hydroxyvinblastine (see later) was isolated only as a minor component (10% yield). In addition, leurosine (3–5% yield) was isolated as a very minor component.

Reaction of **9** with 2 equiv. of osmium tetroxide provided another product (64% yield) which, again on the basis of spectroscopic evidence, was tentatively assigned the name, 3′-hydroxy-19′-oxovinblastine.

More recent studies with several salt derivatives of 3′,4′-dehydrovinblastine provided optimum conditions for obtaining the diol and lactam diol derivatives isolated in the above investigations. Experiments were performed with the hydrochloride and hydrosulfate derivatives, with the latter providing the best results. Thus reaction of the hydrosulfate derivative with 1.2 equiv. of osmium tetroxide afforded a 42% yield of the amine diol, whereas with 2.2 equiv. of this reagent a 63% yield of the lactam diol was obtained. Again leurosine was isolated as a minor component in these reactions.

It is important to emphasize at this point in the discussion that the initial experiments involving *tert*-butyl hydroperoxide, and osmium tetroxide, with 3′,4′-dehydrovinblastine, as reported in preliminary communications (19), were completed prior to our studies (5, 15) which established with certainty the epoxide stereochemistry in leurosine. In those early studies it was assumed that attack of reagents to the 3′,4′-double bond in **7** would occur from the β face of the indole unit. The synthesis of leurosine via the hydroperoxide procedure discussed earlier clearly establishes α attack at the 3′,4′ position in **7**. The isolation of leurosine in the osmylation studies also provides evidence for a similar reaction with osmium tetroxide thereby suggesting stereochemical assignment of the vicinal diol system in **10** and **12** as indicated. On this basis these two compounds are derivatives of leurosidine (**17**, R = CO_2CH_3, R_1 = OH), the C-4′ isomer of vinblastine (16). Conclusive chemical evidence for this stereochemical assignment was obtained in the following manner.

Tosylation of the lactam diol (**12**, R_1 = R_3 = OH, R_2 = O) provided the secondary monotosylate (**12**, R_1 = OH, R_3 = OTos, R_2 = O) and the latter upon reaction with sodium cyanoborohydride afforded leurosine (**8**). This conversion established the α orientation for the tertiary hydroxyl group (R_1) in **12** and, in turn, the same orientation for the secondary function (R_3) since osmylation is well known to be a *cis* hydroxylation process. On this basis the amine diol **10** (R_1 = OH) is given the name, 3′α-hydroxyleurosidine while **12** (R_1 = R_3 = OH, R_2 = O) is 3′α-hydroxy-19′-oxoleurosidine.

The above studies suggested an efficient synthetic entry into a series of novel lactam derivatives in the natural bisindole alkaloid family. The osmylation studies, for example, revealed selective oxidation of the basic nitrogen in the indole unit in the highly functionalized bisindole system and since such intermediates would be important in subsequent investi-

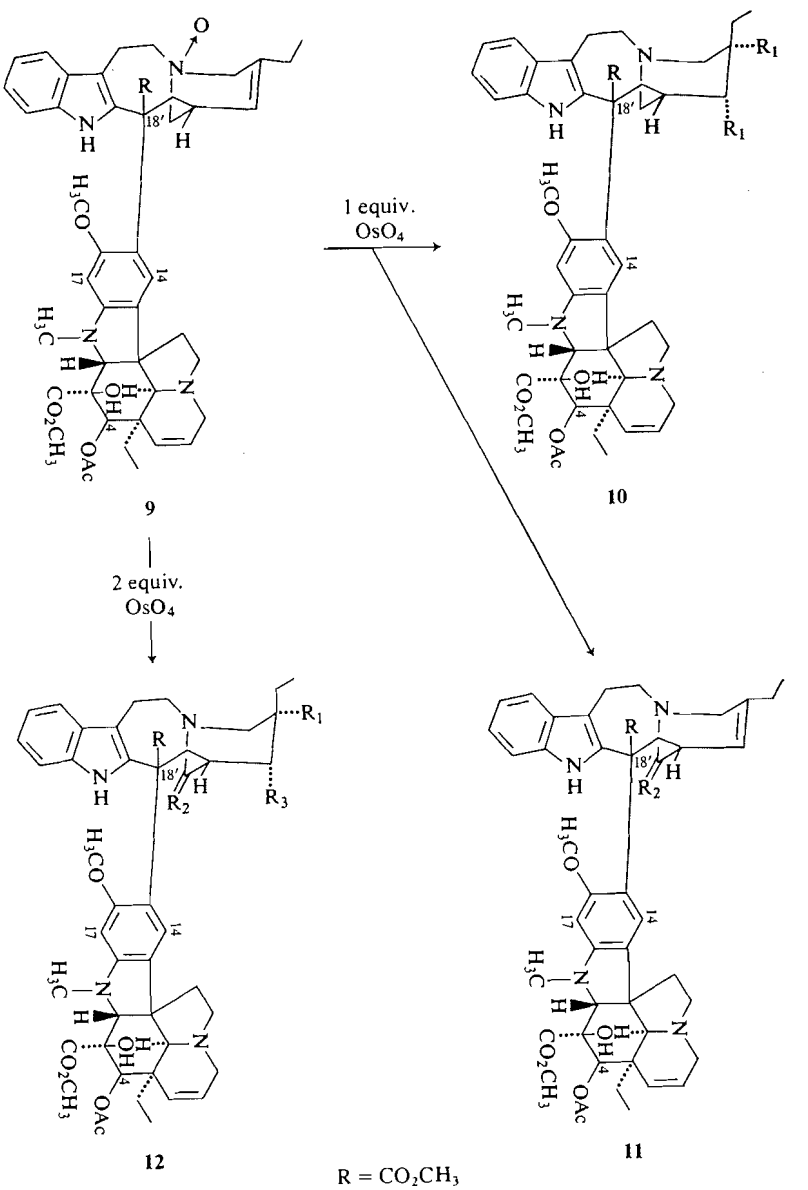


FIG. 1. The reaction of the *N*-oxide intermediate **9** with osmium tetroxide under various conditions.

gations concerned with structure–activity relationships, it was decided to pursue a more thorough investigation in this direction.

We had demonstrated in earlier experiments (4, 17) that conversion of the catharanthine system to the corresponding lactam series could be accomplished by reaction with iodine under basic conditions. The utilization of this reagent with the natural alkaloids vinblastine (**15**, R = CO_2CH_3, R_1 = OH), leurosidine (**17**, R = CO_2CH_3, R_1 = OH), and leurosine (**8**) came under investigation. Figures 2 and 3 provide a summary of the results obtained.

Reaction of vinblastine with iodine and sodium bicarbonate in a tetrahydrofuran solution at room temperature provided 19′-oxovinblastine (**16**, R = CO_2CH_3, R_1 = OH, R_2 = O; 32% yield), whereas a similar oxidation with leurosidine afforded 19′-oxoleurosidine (**18**, R = CO_2CH_3, R_1 = OH, R_2 = O; 62% yield).

The rather unstable alkaloid leurosine can be converted to its lactam derivative **13** (R_1 = O) in 56% yield by means of the iodine oxidation procedure.

The trifluoroacetic acid catalyzed oxygenation

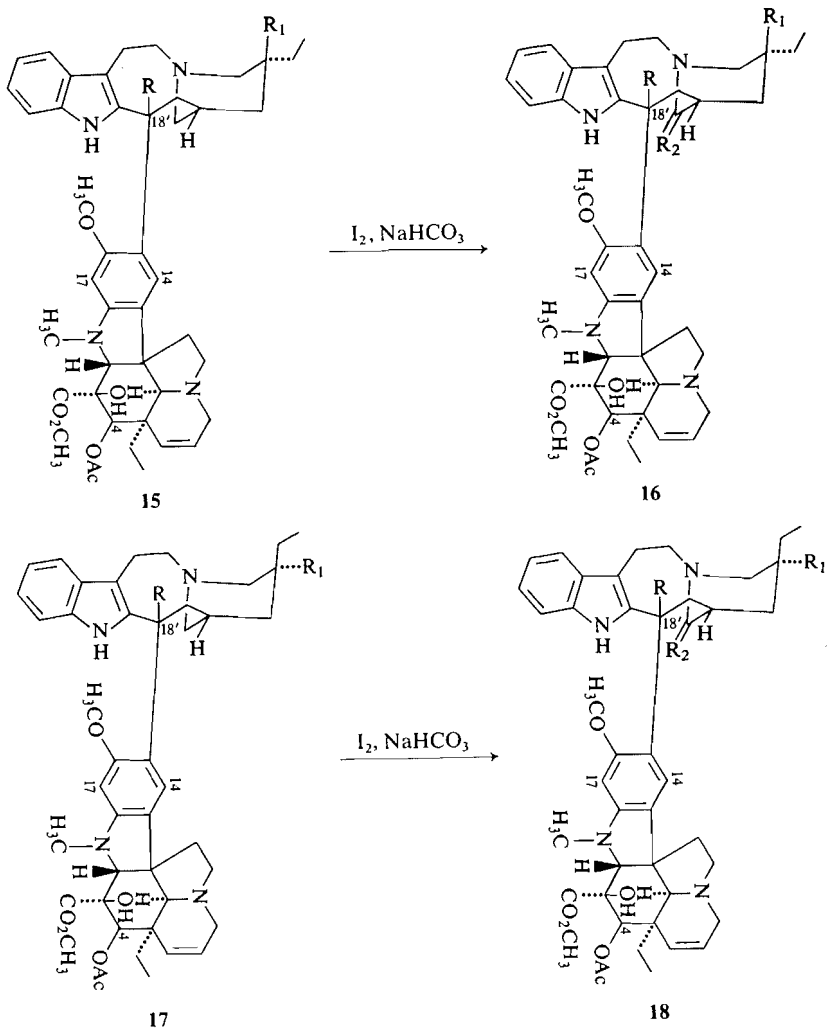


FIG. 2. The conversion of vinblastine (**15**, R = CO_2CH_3, R_1 = OH) and leurosidine (**17**, R = CO_2CH_3, R_1 = OH) to lactam derivatives by means of iodine under basic conditions.

procedure discussed earlier was also evaluated for the purpose of providing lactam derivatives in the bisindole area. It was interesting to observe that with this procedure another series of lactams became available. Thus leurosine was converted to 5′-oxoleurosine (**14**, R_1 = O; 15% yield). A more efficient synthesis of this intermediate (34% yield) involved the direct oxygenation of the synthetic derivative, 3′,4′-dehydrovinblastine (**7**).

In summary, the above investigations with various reagents which allow specific functionalization of the indole unit provided reasonably efficient synthetic entries into a whole series of novel bisindole derivatives. The above synthesis of leurosine, by mercuric acetate oxidation, is the most efficient and most easily adaptable for commercial purposes of those reported (5, 15, 18). Further studies in this direction are currently underway.

Experimental

Melting points were determined on a Kofler block and are uncorrected. Ultraviolet (uv) spectra were recorded on a Cary 15 spectrophotometer in ethanol solution. The wavelengths of absorption maxima are reported in nanometres (nm) with log ε values in parentheses. Infrared (ir) spectra were measured on a Perkin Elmer model 710 or 457 spectrophotometer in chloroform solution. The absorption maxima are reported in wavenumbers (cm^{-1}), calibrated with respect to the absorption band of polystyrene at 1601 cm^{-1}. Proton magnetic resonance (^{1}Hmr) spectra were measured in deuterochloroform ($CDCl_3$) solution at ambient temperature on either a Varian HA-100 or XL-100 spectrometer. Chemical shift values are given in the δ (ppm) scale relative to tetramethylsilane (TMS) used as internal standard. The integrated peak areas, signal multi-

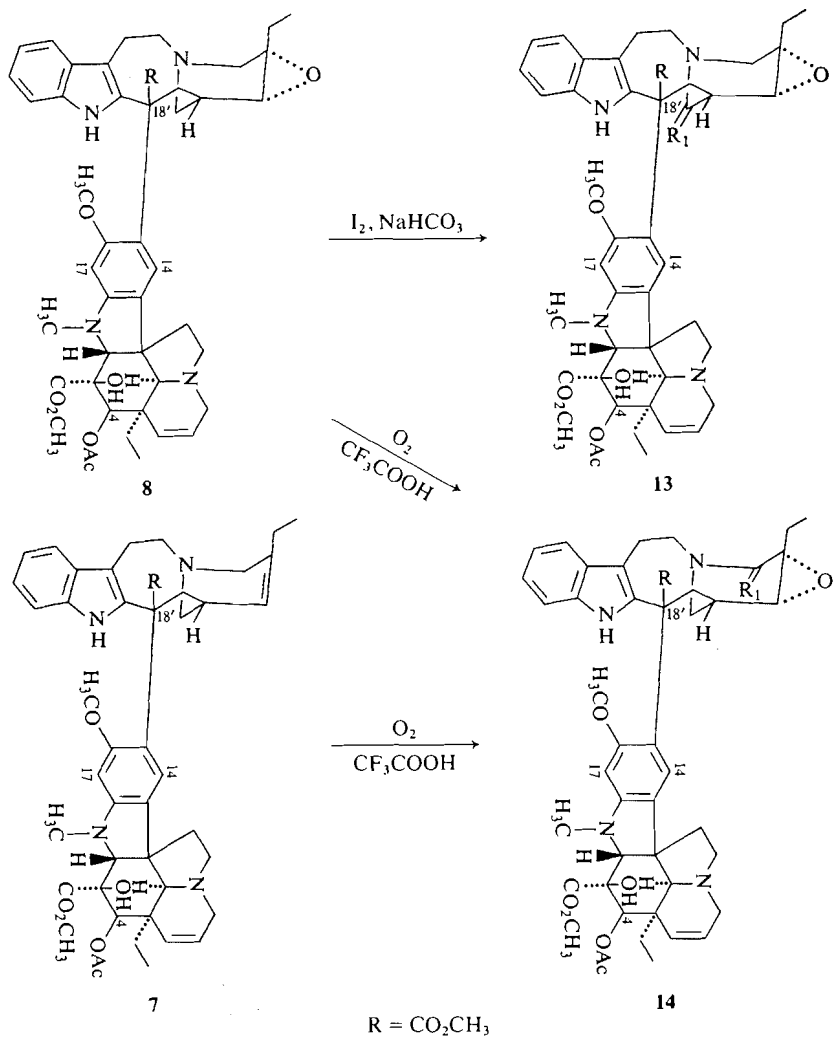


FIG. 3. The conversion of leurosine (**8**) and 3′,4′-dehydrovinblastine (**7**) to lactam derivatives.

plicities and proton assignments are given in parentheses. Low resolution mass spectra (ms) were determined on either an AEI-MS-902 or an Atlas CH-4B spectrometer. High resolution mass spectra were measured on an AEI-MS-902 instrument. Microanalyses were carried out by Mr. P. Borda of the Microanalytical Laboratory, University of British Columbia.

Thin-layer chromatography (tlc) utilized Merck silica gel G (according to Stahl) containing 2% fluorescent indicator. For preparative-layer chromatography (plc), plates (20 × 20 or 20 × 60 cm) of 1 mm thickness were used. Visualization was effected by viewing under ultraviolet light and/or by colour reaction with ceric sulphate spray reagent. Column chromatography utilized Merck silica gel 60 (70–230 mesh) or Merck aluminum oxide 90 (neutral).

As a matter of routine, all reagents and solvents were recrystallized or distilled before use.

N_a-Carbomethoxy-18β-carbomethoxycleavamine (1)

A solution of 18β carbomethoxycleavamine (502 mg, 1.5 mmol) in dry tetrahydrofuran (50 ml) under an atmosphere of nitrogen was treated with a suspension of potassium hydride in mineral oil (300 mg, 22.5% KH, 1.7 mmol). After stirring for 10 min at ambient temperature the resultant mixture was treated with methyl chloroformate (290 mg, 3.1 mmol) and stirred for a further 1 h. Acetic acid (0.5 ml) was added to the mixture and stirring continued for 5 min. The solvent was removed *in vacuo* and the residue chromatographed on alumina (activity III, petroleum ether – benzene) to give N_a-carbomethoxy-18β-carbomethoxycleavamine (**1**) (461 mg, 78%), mp 124–125°C (acetone–ether); uv λ_{max}: 294 (3.66), 283 (3.80), 268 (4.09), 262 (4.11), 228 (4.37); ir ν_{max}: 1725; ^{1}Hmr δ: 8.08 (1H, m, C_{14}-*H*) 7.23 (3H, m, C_{11}-*H*, C_{12}-*H*, C_{13}-*H*), 5.77 (1H, d, J = 6 Hz, C_{18}-*H*), 5.37 (1H, m, C_3-*H*), 3.88 (3H, s, N—CO_2CH_3), 3.53 (3H, s, —CO_2CH_3), 0.98 (3H, t, J = 7 Hz, —CH_2CH_3); ms *m/e* 396 (M^+), 273 (base peak), 136, 124; ^{13}Cmr δ: 12.66 (C-21), 122.25 (C-3), 141.11 (C-4). High resolution molecular weight determination calcd. for $C_{23}H_{28}N_2O_4$: 396.2048; found: 396.2070. *Anal.* calcd. for $C_{23}H_{28}N_2O_4$: C 69.67, H 7.12, N 7.07; found: C 69.86, H 7.10, N 6.87.

Autoxidation of N_a*-Carbomethoxy-18β-carbomethoxycleavamine (1)*

A solution of N_a-carbomethoxy-18β-carbomethoxycleavamine (**1**) (400 mg, 1 mmol) in peroxide-free tetrahydrofuran (10 ml) containing aqueous 1% trifluoroacetic acid (1 ml) was stirred at ambient temperature in the presence of air for a period of 8 days. After drying (K_2CO_3) the solvent was removed *in vacuo* to give an amber gum. Chromatography on alumina (activity III) afforded the *epoxide* **2** (40 mg, 10%) and the *epoxy lactam* **3** (230 mg, 54%).

N_a-Carbomethoxy-18β-carbomethoxy-3β,4β-epoxydihydrocleavamine (**2**), mp 131–132°C (ether); uv λ_{max}: 294 (3.66), 283 sh (3.76), 268 (4.08), 262 (4.09), 227 (4.32); ir ν_{max}: 1728; ^{1}Hmr δ: 8.1 (1H, m, C_{14}-*H*), 7.3 (3H, m, C_{11}-*H*, C_{12}-*H*, C_{13}-*H*), 5.84 (1H, d, *J*=6 Hz, C_{18}-*H*), 3.91 (3H, s, N—CO_2CH_3), 3.57 (3H, s, —CO_2CH_3), 1.00 (3H, t, *J* = 7.5 Hz, —CH_2CH_3); ms *m/e* 412 (M^+), 224 (base peak), 152, 138; ^{13}Cmr δ: 8.93 (C-21), 60.6 (C-3), 62.7 (C-4). High resolution molecular weight determination calcd. for $C_{23}H_{28}N_2O_5$: 412.1997; found: 412.2027. *Anal.* calcd. for $C_{23}H_{28}N_2O_5$: C 66.97, H 6.84, N 6.79; found: C 66.81, H 6.87, N 6.71.

N_a-Carbomethoxy-18β-carbomethoxy-5-oxo-3β,4β-epoxydihydrocleavamine (**3**), obtained as a foam from methanol; uv λ_{max}: 293 (3.67), 281 sh (3.78), 265 sh (4.05), 259 (4.06), 227 (4.38); ir ν_{max}: 1732, 1680, 1650; ^{1}Hmr δ: 8.1 (1H, m, C_{14}-H), 7.3 (3H, m, C_{11}-*H*, C_{12}-*H*, C_{13}-*H*), 5.23 (1H, m), 4.00 (3H, bs, N—CO_2CH_3), 3.66 (3H, s, —CO_2CH_3), 1.90 (2H, q, *J* = 7 Hz, —CH_2CH_3), 0.79 (3H, t, *J* = 7 Hz, —CH_2CH_3); ms *m/e* 426 (M^+, base peak), 394, 228. High resolution molecular weight determination calcd. for $C_{23}H_{26}N_2O_6$: 426.1791; found: 426.1795. *Anal.* calcd. for $C_{23}H_{26}N_2O_6 \cdot \frac{1}{2}CH_3OH$:[3] C 63.80, H 6.33, N 6.33; found: C 64.08, H 6.11, N 6.31.

Oxidation of N_a*-Carbomethoxy-18β-carbomethoxycleavamine (1) using* tert*-Butyl Hydroperoxide*

A solution of **1** (1.76 g, 4.4 mmol) in freshly distilled tetrahydrofuran (50 ml) containing aqueous 1% trifluoroacetic acid (10 ml) and *tert*-butyl hydroperoxide (9 ml) was stirred at ambient temperature for 21 h. The mixture was taken up in $NaHCO_3$ solution (40 ml) and extracted with ethyl acetate (2 × 30 ml). The combined organic portion was washed with 5% NaOH solution (1 × 20 ml), water (1 × 20 ml), and brine (1 × 20 ml). After drying (Na_2SO_4) the solvent was removed *in vacuo* to give a viscous oil. Chromatography on alumina (activity III, φH) afforded the epoxide **2** (1.22 g, 67%) identical with that obtained above.

Reduction of N_a*-Carbomethoxy-18β-carbomethoxy-3β,4β-epoxydihydrocleavamine*

A solution of **2** (25 mg, 0.06 mmol) in dry *N*-methylmorpholine (2.5 ml) under an atmosphere of nitrogen was treated with lithium aluminum hydride (75 mg, 2 mmol) in tetrahydrofuran (0.5 ml). The tetrahydrofuran was distilled out of the reaction mixture and the remaining solution was refluxed for 2 h. Excess lithium aluminum hydride was destroyed by careful addition of water (10 ml). The mixture was extracted with ethyl acetate (4 × 5 ml), the combined organic portion was dried (Na_2SO_4), and the solvents were removed *in vacuo*. Chromatography on silica gel (ethyl acetate) afforded the *diol* **4** (R = H) (16 mg, 80%), mp 145–150°C (methanol); uv λ_{max}: 291 (3.81), 283 (3.84), 275 sh (3.79), 228 (4.51); ir ν_{max}: 3600, 1510, 1025; ^{1}Hmr δ: 8.55 (1H, bs, N—*H*), 7.51 (1H, m, C_{14}-*H*), 7.24 (3H, m, C_{11}-*H*, C_{12}-*H*, C_{13}-*H*), 4.24 (1H, quintet, *J* = 5 Hz, C_{18}-*H*), 3.86 (2H, d, *J* = 5 Hz, —CH_2OH), 0.96 (3H, t, *J* = 7 Hz, —CH_2CH_3); ms *m/e* 328 (M^+) 311, 310, 299, 297, 187, 154 (base peak). High resolution molecular weight determination calcd. for $C_{20}H_{28}N_2O_2$: 328.2150; found: 328.2146. *Anal.* calcd. for $C_{20}H_{28}N_2O_2 \cdot \frac{1}{2}CH_3OH$:[3] C 71.48, H 8.78, N 8.13; found: C 71.36, H 8.71, N 8.10.

Reduction of 4β,18-Dihydroxy-18-hydroxymethyldihydrocleavamine (4, R = OH) to 4β-Hydroxy-18β-hydroxymethyldihydrocleavamine

A solution of the triol **4** (R = OH) (37 mg, 0.11 mmol) in *N*-methylmorpholine (3 ml) was treated with lithium aluminum hydride (110 mg, 3 mmol) in tetrahydrofuran (0.75 ml) as described above. Isolation of the product by preparative tlc (silica, ethyl acetate) afforded the diol **4** (R = H) (33 mg, 93%). The physical (tlc, mixture mp) and spectral properties (ir, ^{1}Hmr, ms) showed the product to be identical with the one obtained above.

Synthesis of Leurosine (8) from 3′,4′-Dehydrovinblastine (7) using tert*-Butyl Hydroperoxide*

A solution of 7 (81 mg, 0.1 mmol) in peroxide-free tetrahydrofuran (4 ml) containing aqueous 1% trifluoroacetic acid (0.4 ml) and *tert*-butyl hydroperoxide (0.4 ml) was stirred at ambient temperature for 9 h. The mixture was poured into ice-water (15 ml), made basic with concentrated NH_4OH, and extracted with dichloromethane (3 × 10 ml). The combined organic portion was washed with 3% NaOH solution (1 × 8 ml), dried ($MgSO_4$), and the solvent was removed *in vacuo*. Chromatography on silica gel (ethyl acetate – 10% methanol) afforded leurosine (**8**) (43 mg, 52%) which was identified by comparison with an authentic sample (tlc, ^{1}Hmr, ir, ms, and mixture mp).

Synthesis of Leurosine (8) from 7 using Mercuric Acetate

A solution of the hydrochloride salt of 7 (20 mg) in tetrahydrofuran (1 ml) and water (1 ml) was added at ambient temperature to a suspension of mercuric acetate (16 mg) in tetrahydrofuran (1 ml) and water (1 ml) with stirring. The mixture was stirred for a further 30 min then quenched with 3 *N* NaOH (0.5 ml) and sodium borohydride (excess). After 2 h at ambient temperature the mixture was saturated with sodium chloride and extracted with chloroform. The extract was dried (Na_2SO_4), evaporated, and chromatographed on silica gel to give leurosine (12 mg, 62%) identical with an authentic sample.

The N*-Oxide Derivative (9) of 3′,4′-Dehydrovinblastine*

A solution of 3′,4′-dehydrovinblastine (7) (100 mg, 0.126 mmol) in dichloromethane (5 ml) was cooled to −4°C. *m*-Chloroperbenzoic acid (21.8 mg, 0.126 mmol) was added and the mixture stirred at ca. −4°C for 10 min. Then the solvent was evaporated *in vacuo*. The residue was chromatographed on alumina (grade III, ca. 10 g). Elution with 3:1 ethyl acetate – methanol afforded the *N*-oxide derivative (**9**) (100.7 mg, 99%); mp 194–197°C (methanol); uv λ_{max}: 306 (3.99), 292 (4.24), 283 (4.33), 265 (4.46), 213 (4.98), ir ν_{max}: 3460, 2950, 1735, 1610; ^{1}Hmr δ: 9.76 (1H, bs, O*H*), 8.15 (1H, bs, N*H*), 7.67 (1H, m, $C_{14'}$-*H*), 7.11 (3H, m, $C_{11'}$–$C_{13'}$-*H*'s), 6.43 (1H, s, C_{14}-*H*), 6.10 (1H, s, C_{17}-*H*), 5.83 (1H, m, $C_{7'}$-*H*), 5.39 (1H, m, $C_{3'}$-*H*), 5.39 (1H, s, C_4-*H*), 5.24 (1H, d, *J* = 10 Hz, C_6-*H*), 4.81–3.57 (6H, m, $C_{5'}$, $C_{7'}$, $C_{19'}$-*H*'s), 3.81 (3H, s, OCH_3), 3.77 (3H, s, OCH_3), 3.67 (3H, s, OCH_3), 2.69 (3H, s, NCH_3), 2.07 (3H, s, $OCOCH_3$), 1.01 (3H, t, *J* = 7 Hz, —CH_2CH_3), 0.61 (3H, t, *J* = 7 Hz, —CH_2CH_3); ms *m/e* 808 (M^+), 792, 610, 136, 135, 122, 121, 120, 107, 106 (base peak). High resolution molecular weight determination calcd. for $C_{46}H_{56}N_4O_9$: 808.4046; found: 808.3995.

[3]For analytical purposes, complete removal of solvent of crystallization was impossible without decomposition of the compounds.

Osmylation of the N-*Oxide Derivative (**9**) of 3′,4′-Dehydrovinblastine*

(a) With One or Less than One Equivalent of Osmium Tetroxide

A suspension of the *N*-oxide **9** (100 mg, 0.124 mmol) in dry tetrahydrofuran (4 ml) and pyridine (1 ml) was cooled to ca. −5°C under an atmosphere of argon. Osmium tetroxide (31.4 mg, 0.124 mmol) in dry tetrahydrofuran (0.4 ml) was added and the mixture stirred at −5°C for 45 min. Then hydrogen sulfide was bubbled through the mixture for 5 min. The resultant mixture was filtered and the filtrate evaporated *in vacuo*. The oily product was purified by preparative-layer chromatography (silica gel, 19:1 dichloromethane–methanol).

The major product (least polar on tlc) (53 mg, 53%) was identified as 3′,4′-dehydro-19′-oxovinblastine (**11**, R_2 = O), mp 210–212°C (methanol); uv λ_{max}: 293 (4.47), 283 (4.53), 262 (4.63), 213 (5.17); ir ν_{max}: 3460, 2950, 1735, 1655, 1610; ^{1}Hmr δ: 8.10 (1H, bs, N*H*), 7.60 (1H, m, $C_{14'}$-*H*), 7.21 (3H, m, $C_{11'}$–$C_{13'}$-*H*'s), 6.69 (1H, s, C_{14}-*H*), 6.19 (1H, s, C_{17}-*H*), 6.15 (1H, m, $C_{3'}$-*H*), 5.90 (1H, m, $C_{7'}$-*H*), 5.52 (1H, s, C_4-*H*), 5.34 (1H, d, J = 10 Hz, C_6-*H*), 4.92 (1H, m, $C_{2'}$-*H*), 3.84 (6H, bs, 2 × OC*H*$_3$), 3.79 (1H, s, C_2-*H*), 3.62 (3H, s, OC*H*$_3$), 2.75 (3H, s, NC*H*$_3$), 2.13 (3H, s, OCOC*H*$_3$), 1.01 (3H, t, J = 7 Hz, —CH_2CH_3), 0.85 (3H, t, J = 8 Hz, —CH_2CH_3); ms *m/e* 806 (M^+), 748, 670, 136, 135 (base peak), 122, 121, 107, 106. High resolution molecular weight determination calcd. for $C_{46}H_{54}N_4O_9$: 806.3890; found: 806.3873. *Anal.* calcd. for $C_{46}H_{54}N_4O_9 \cdot CH_3OH$: C 67.28, H 6.97, N 6.68; found: C 66.98, H 6.80, N, 6.43.

The second product (more polar on tlc) was leurosine (**8**, R = CO_2CH_3) (3.6 mg, 4%). The identity of this compound was established by tlc mobility, spectral comparison (^{1}Hmr, ms), and mixture melting point determination with an authentic sample.

The third product (most polar on tlc) was 3′α-hydroxyleurosidine (**10**, R_1 = OH; formerly designated '3′-hydroxyvinblastine') (9.4 mg, 9%); uv λ_{max}: 294 (4.37), 285 (4.40), 258 (4.49), 212 (5.00); ir ν_{max}: 3550, 3460, 2960, 1735, 1610; ^{1}Hmr δ: 8.00 (1H, bs, N*H*), 7.50 (1H, m, $C_{14'}$-*H*), 7.15 (3H, m, $C_{11'}$–$C_{13'}$-*H*'s), 6.60 (1H, s, C_{14}-*H*), 6.13 (1H, s, C_{17}-*H*), 5.86 (1H, m, $C_{7'}$-*H*), 5.48 (1H, s, C_4-*H*), 5.30 (1H, d, J = 10 Hz, C_6-*H*), 3.82 (3H, s, OC*H*$_3$), 3.80 (3H, s, OC*H*$_3$), 3.75 (1H, s, C_2-*H*), 3.63 (3H, s, OC*H*$_3$), 2.72 (3H, s, NC*H*$_3$), 2.10 (3H, s, OCOC*H*$_3$), 0.96 (3H, t, J = 7 Hz, —CH_2CH_3), 0.90 (3H, t, J = 8 Hz, —CH_2CH_3); ms *m/e* 826 (M^+), 809, 285 (base peak), 136, 135, 122, 121, 107, 106. High resolution molecular weight determination calcd. for $C_{46}H_{58}N_4O_{10}$: 826.4153; found: 826.4178. The hydrochloride salt had mp > 200°C (dec.). *Anal.* calcd. for $C_{49}H_{59}N_4O_{10}Cl$: C 63.98, H 6.89, N 6.50; found: C 64.18, H 6.85, N 6.17.

(b) With Two Equivalents of Osmium Tetroxide

A suspension of the *N*-oxide **9** (50 mg, 0.062 mmol) in dry tetrahydrofuran (2 ml) and pyridine (0.5 ml) was cooled to 0°C under an atmosphere of argon. Osmium tetroxide (32 mg, 0.126 mmol) in dry tetrahydrofuran (0.4 ml) was added and the mixture stirred at ca. 0°C for 1.5 h. Hydrogen sulfide was then bubbled through the mixture for 5 min. The resultant mixture was filtered and the filtrate evaporated *in vacuo*. The crude product was purified by preparative-layer chromatography (silica gel, 23:2 dichloromethane–methanol).

The major product (33.7 mg, 65%) was 3′α-hydroxy-19′-oxoleurosidine (**12**, R_1 = R_3 = OH, R_2 = O; formerly designated '3′-hydroxy-19′-oxovinblastine'), mp 227–229°C; uv λ_{max}: 293 (4.33), 283 (4.39), 261 (4.45), 213 (5.01); ir ν_{max}: 3600–3500, 3460, 2960, 1735, 1645, 1615; ^{1}Hmr δ: 8.04 (1H, bs, N*H*), 7.54 (1H, m, $C_{14'}$-*H*), 7.18 (3H, m, $C_{11'}$–$C_{13'}$-*H*'s), 6.66 (1H, s, C_{14}-*H*), 6.17 (1H, s, C_{17}-*H*), 5.89 (1H, m, $C_{7'}$-*H*), 5.53 (1H, s, C_4-*H*), 5.33 (1H, d, J = 10 Hz, C_6-*H*), 4.65 (1H, m, $C_{2'}$-*H*), 3.83 (6H, bs, 2 × OC*H*$_3$), 3.81 (1H, s, C_2-*H*), 3.60 (3H, s, OC*H*$_3$), 2.76 (3H, s, NC*H*$_3$), 2.12 (3H, s, OCOC*H*$_3$), 1.04 (3H, t, J = 7 Hz, —CH_2CH_3), 0.85 (3H, t, J = 7 Hz, —CH_2CH_3); ms *m/e* 840 (M^+), 423, 282, 149, 135 (base peak), 122, 121, 107. High resolution molecular weight determination calcd. for $C_{46}H_{56}N_4O_{11}$: 840.3945; found: 840.3904. *Anal.* calcd. for $C_{46}H_{56}N_4O_{11}$: C 65.70, H 6.71, N 6.66; found: C 65.45, H 6.61, N 6.51.

*3′α-Hydroxyleurosidine (**10**, R_1 = OH)*

Osmium tetroxide (36 mg, 0.14 mmol) in dry tetrahydrofuran (0.5 ml) was added to the hydrochloride salt of 3′,4′-dehydrovinblastine 7, (111 mg, 0.135 mmol) in dry tetrahydrofuran (2 ml) at 0°C under an atmosphere of dry nitrogen. After 15 min dry pyridine (0.03 ml, 0.0294 g, 0.372 mmol) was added and stirring continued at 0°C for 2 h. Hydrogen sulphide was bubbled through the solution for 5 min, the mixture diluted with ethyl acetate, washed with dilute ammonia solution, dried (Na_2SO_4) and concentrated *in vacuo*. Chromatography on silica gel gave **10** (R_1 = OH), 25 mg (23%), identical with that obtained earlier.

*3′α-Hydroxy-19′-oxoleurosidine (**12**, R_1 = R_3 = OH, R_2 = O) and 3′α-Hydroxyleurosidine (**10**, R_1 = OH)*

(*a*) Osmylation of the hydrosulfate salt of 7 with osmium tetroxide (2.2 equiv.) in dry tetrahydrofuran at 0°C as described directly above afforded the lactam diol (**12**, R_1 = R_3 = OH, R_2 = O) (63%) and the diol (**10**, R_1 = OH) (12%). These products were identical with the respective authentic samples.

(*b*) Osmylation as in (*a*), using 1.2 equiv. of osmium tetroxide gave the diol (**10**, R_1 = OH) (42%).

*The Tosylate (**12**, R_1 = OH, R_3 = OTos, R_2 = O)*

Toluenesulfonyl chloride (10 mg) was added to a solution of the lactam diol (**12**, R_1 = R_3 = OH, R_2 = O) (18 mg) in dry pyridine (2 ml). The mixture was heated at 40°C for 4 h, allowed to stand at ambient temperature for 16 h, poured into water, and extracted with chloroform. The extracts were combined, dried (Na_2SO_4) and concentrated *in vacuo*. Chromatography on silica gel afforded the monotosylate (6 mg, 63%); mp 218–220°C; uv λ_{max}: 312 (sh, 3.88), 295 (4.18), 286 (4.23), 272 (4.27), and 265 (4.27); ir ν_{max}: 3475, 1730, 1660, 1360; ^{1}Hmr δ: 8.68 (1H, bs, —N*H*), 7.73 (2H, d, J = 8 Hz, Tos protons), 7.60 (1H, m, $C_{14'}$-*H*), 7.41 (2H, d, J = 8 Hz, Tos protons), 7.24 (3H, m, indole aromatic protons), 6.71 (1H, s, C_{14}-*H*), 6.27 (1H, s, C_{17}-*H*), 5.96 (1H, dd, J = 10, 4 Hz, $C_{7'}$-*H*), 5.57 (1H, s, C_4-*H*), 5.36 (1H, d, J = 10 Hz, C_6-*H*), 4.72 (1H, bd, J = 13 Hz, $C_{2'}$-*H*), 4.22 (1H, bs, C*H*—OTos), 3.97 (3H, s, —OC*H*$_3$), 3.86 (3H, s, —OC*H*$_3$), 3.70 (3H, s, —OC*H*$_3$), 2.81 (3H, s, —NC*H*$_3$), 2.53 (3H, s, Ph—C*H*$_3$), 2.17 (3H, s, —OCOC*H*$_3$). *Anal.* calcd. for $C_{53}H_{62}N_4O_{13}S$: C 63.86, H 6.28, N 5.63; found: C 63.81, H 6.40, N 5.25.

Starting material (10 mg) was recovered.

*Leurosine Lactam (**13**, R_1 = O) from the Monotosylate*

Excess sodium cyanoborohydride was added to a solution of **12** (R_1 = OH, R_2 = O, R_3 = OTos) (10 mg) in dry HMPA (2 ml). The mixture was heated at 90°C for 20 h, poured into water and extracted with ethyl acetate. The organic layer was washed with water, dried (Na_2SO_4), and concentrated *in vacuo*. Chromatography on silica gel afforded **13** (R_1 = O) (2.5 mg, 30%), identical with an authentic sample.

*19′-Oxovinblastine (**16**, R = CO_2CH_3, R_1 = OH, R_2 = O)*

Iodine (20 mg) was added to a mixture of vinblastine (**15**, R = CO_2CH_3, R_1 = OH) (18 mg) and sodium bicarbonate

(20 mg) in aqueous 70% tetrahydrofuran (4 ml). The mixture was stirred at ambient temperature for 10 min, diluted with saturated sodium bicarbonate solution (4 ml), and extracted with dichloromethane. The organic phase was washed with 10% sodium bisulphite solution, dried (Na_2SO_4), and concentrated *in vacuo*. Chromatography on silica gel gave **16** (R = CO_2CH_3, R_1 = OH, R_2 = O) (6 mg, 33%) as a foam from methanol; uv λ_{max}: 311 (sh, 3.74), 294 (3.99), 285 (4.02), 259 (4.14), 213 (4.69); ir ν_{max}: 3475, 1740, 1640; ^{1}Hmr δ: 8.06 (1H, bs, —N—*H*), 7.54 (1H, m, $C_{14'}$-*H*), 7.20 (3H, m, $C_{11',12',13'}$-*H*), 6.68 (1H, s, C_{14}-*H*), 6.18 (1H, s, C_{17}-*H*), 5.91 (1H, dd, *J* = 3.5, 10.5 Hz, C_7-*H*), 5.54 (1H, s, C_4-*H*), 5.34 (1H, d, *J* = 10.5 Hz, C_6-*H*), 4.70 (1H, m, $C_{2'}$-*H*), 3.82 (3H, s, —CO_2CH_3), 3.80 (3H, s, —CO_2CH_3), 3.60 (3H, s, —OCH_3), 2.75 (3H, s, —NCH_3), 2.12 (3H, s, —$OCOCH_3$), 0.87 (6H, bt, *J* = 7.5 Hz, 2 × —CH_2CH_3); ms *m/e* 824 (M^+), 765, 665, 135 (base peak). High resolution molecular weight determination calcd. for $C_{46}H_{56}N_4O_{10}$: 824.3996; found: 824.3971. *Anal.* calcd. for $C_{46}H_{56}N_4O_{10}\cdot CH_3OH$:[3] C 65.89, H 7.01, N 6.54; found: C 65.14, H 7.20, N 6.60.

*19'-Oxoleurosidine (**18**, R = CO_2CH_3, R_1 = OH, R_2 = O)*

Oxidation of leurosidine (**17**, R = CO_2CH_3, R_1 = OH), as described above for the oxidation of vinblastine, gave the lactam (**18**, R = CO_2CH_3, R_1 = OH, R_2 = O) (62%) as a clear glass mp 196–210°C; uv λ_{max}: 309 (sh, 3.74), 294 (4.00), 285 (4.04), 262 (4.11), 214 (4.66); ir ν_{max}: 3477, 1738, 1644; ^{1}Hmr δ: 8.07 (1H, bs, —N*H*), 7.51 (1H, m, $C_{14'}$-*H*), 7.16 (3H, m, $C_{11',12',13'}$-*H*), 6.63 (1H, s, C_{14}-*H*), 6.16 (1H, s, C_{17}-*H*), 5.88 (1H, dd, *J* = 10, 4 Hz, C_7-*H*), 5.50 (1H, s, C_4-*H*), 5.32 (1H, bd, *J* = 10 Hz, C_6-*H*), 4.73 (1H, m, $C_{2'}$-*H*), 3.82 (6H, s, 2 × —CO_2CH_3), 3.60 (3H, s, —OCH_3), 2.74 (3H, s, —NCH_3), 2.11 (3H, s, —$OCOCH_3$), 1.17 (3H, t, *J* = 7.5 Hz, —CH_2CH_3), 0.96 (3H, t, *J* = 7.5 Hz, —CH_2CH_3); ms *m/e* 824 (M^+), 765, 282, 135 (base peak). High resolution molecular weight determination calcd. for $C_{46}H_{56}N_4O_{10}$: 824.3996; found: 824.3953.

*19'-Oxoleurosine (**13**, R = CO_2CH_3, R_1 = O)*

Oxidation of leurosine (**8**, R = CO_2CH_3) as described above gave the lactam (**13**, R = CO_2CH_3, R_1 = O) (56%) as a foam from methanol; uv λ_{max}: 309 (sh, 3.74), 294 (4.00), 284 (4.05), 262 (4.13), 214 (4.66); ir ν_{max}: 3470, 1738, 1644; ^{1}Hmr δ: 8.06 (1H, bs, —N*H*), 7.57 (1H, m, $C_{14'}$-*H*), 7.18 (3H, m, $C_{11',12',13'}$-*H*), 6.65 (1H, s, C_{14}-*H*), 6.19 (1H, s, C_{17}-*H*), 5.90 (1H, dd, *J* = 10.5, 3.5 Hz, C_7-*H*), 5.51 (1H, s, C_4-*H*), 5.33 (1H, bd, *J* = 10.5 Hz, C_6-*H*), 4.76 (1H, m, $C_{2'}$-*H*), 3.85 (3H, s, —CO_2CH_3), 3.83 (3H, s, —CO_2CH_3), 3.63 (3H, s, —OCH_3), 3.04 (1H, d, *J* = 3.5 Hz, $C_{3'}$-*H*), 2.76 (3H, s, —NCH_3), 2.12 (3H, s, —$OCOCH_3$), 1.01 (3H, t, *J* = 7.5 Hz, —CH_2CH_3), 0.84 (3H, t, *J* = 7.5 Hz, —CH_2CH_3); ms *m/e* 822 (M^+), 763, 282, 135 (base peak). High resolution molecular weight determination calcd. for $C_{46}H_{54}N_4O_{10}$: 822.3839; found: 822.3806. *Anal.* calcd. for $C_{46}H_{54}N_4O_{10}\cdot CH_3OH$:[3] C 66.04, H 6.80, N 6.56; found: C 65.26, H 6.91, N 6.76.

*5'-Oxoleurosine (**14**, R_1 = O)*

(*a*) A solution of leurosine (**8**) (30 mg) in tetrahydrofuran (2 ml) containing aqueous 1% trifluoroacetic acid (0.2 ml) was stirred in the presence of air for 11 days. The mixture was diluted with saturated sodium bicarbonate solution (5 ml) and extracted with dichloromethane. The organic layer was dried (Na_2SO_4) and concentrated *in vacuo*. Chromatography on silica gel gave leurosine (4 mg) and the lactam (**14**, R_1 = O) (4 mg, 15%) as a clear glass, mp 185–191°C; uv λ_{max}: 307 (3.91), 290 (4.04), 283 (4.06), 263 (4.15), 215 (4.70); ir ν_{max}: 3425, 1738, 1681, 1652; ^{1}Hmr δ: 8.16 (1H, bs, —N*H*), 6.88 (1H, s, C_{14}-*H*), 5.99 (1H, s, C_{17}-*H*), 5.32 (1H, bd. *J* = 10.5 Hz, C_6-*H*), 3.80 (6H, s, 2 × —OCH_3), 3.70 (3H, s, —OCH_3), 2.68 (3H, s, —NCH_3), 2.10 (3H, s, —$OCOCH_3$), 0.70 (3H, t, *J* = 7.5 Hz, —CH_2CH_3), 0.40 (3H, t, *J* = 7.5 Hz, —CH_2CH_3); ms *m/e* 822 (M^+), 662, 149, 135 (base peak). High resolution molecular weight determination calcd. for $C_{46}H_{54}N_4O_{10}$: 822.3839; found: 822.3915. *Anal.* calcd. for $C_{46}H_{54}N_4O_{10}$: C 67.15, H 6.57, N 6.81; found: C 66.51, H 6.55, N 6.87.

(*b*) Oxidation of (**7**) as described above in (*a*) gave the lactam (**14**, R_1 = O), (34%), identical with that obtained earlier.

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