Tetrahedron Vol. 36, pp. 1057 to 1062
Pergamon Press Ltd., 1980. Printed in Great Britain

THE TOTAL SYNTHESIS OF (+)-16-HYDROXYDIHYDROCLEAVAMINE AND THE PARTIAL SYNTHESIS OF DEMETHOXYCARBONYLDEOXYVINBLASTINE†

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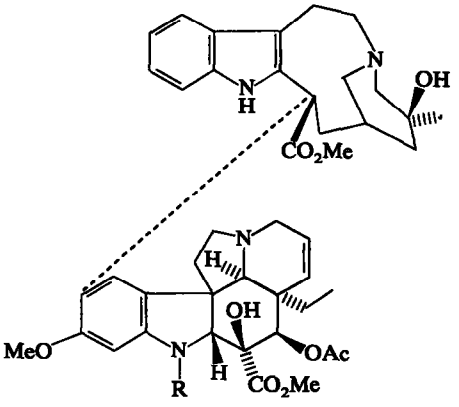
Received in U.K. 5 *February* 1979

Abstract—The total synthesis of (+)-16-hydroxydihydrocleavamine and the partial synthesis of demethoxycarbonyldeoxyvinblastine are described.

The binary indole alkaloids, vinblastine (**1**) and vincristine (**2**) are considered to be among the most active chemotherapeutic agents against a variety of cancers including Hodgkins disease and acute leukaemia in children. We have previously described the first syntheses of (+)-α-and β-dihydrocleavamines and 16-methoxycarbonyldihydrocleavamine which contained the carbon skeleton of the indole moiety of vinblastine. In



(**1**): R = CH_3
(**2**): R = CHO

order to convert these substances to vinblastine derivatives it was necessary to activate them at C-16 for attack by vindoline. The lactam ester (**3**) used in the synthesis of (±)-α- and β-dihydrocleavamines (**10**)[1] was chosen as a starting point for the synthesis of (±)-16-hydroxydihydrocleavamine. On treatment of the lactam (**3**) with an excess of phosphorus pentasulphide in dry xylene at 100°, tlc showed complete conversion to the thio-compound (**4**). On work up a crystalline white solid, m.p. 156° was obtained in 80% yield. The compound possessed an indolic UV and the IR spectrum indicated the absence of CO stretching vibrations for the lactam CO functions. Instead, the absorptions at 1597 cm^{-1} for the thioamide II band were distinguishable. The mass spectrum showed the presence of the molecular ion at *m/e* 356 and the expected fragmentation pattern.

Initial attempts at desulphurisation of the thiolactam (**4**) with Raney nickel failed to give the desired product. It was found that in most solvents a new compound was formed which gave a fluorescent base spot on tlc. After work up this compound was isolated as a gum which showed the absence of thioamide II band in its IR spectrum. The most peculiar feature of this by-product was its non-indolic UV spectrum which was reminiscent of the UV spectrum of alstonine-type alkaloids in which ring C is aromatized suggesting that aromatization of C might have occurred. After considerable variation of reaction parameters it was found that when 80% ethanol was used as the solvent and when the reaction temperature was kept at 40°, complete conversion to another indolic compound occurred within 15 min of the addition of W-6 Raney nickel. The same reaction could also be carried out in 80% ethanol at 25° for 35 min without the formation of the non-indolic by product. These conditions were found to be critical, any further increase in reaction time leading to the formation of the fluorescent by-product. On work-up a colourless gum was obtained in over 80% yields which afforded a crystalline indolic methiodide, m.pt. 188°, which showed the presence of an ester CO but the absence of the thioamide bands in its IR spectrum. The mass spectrum of the free base (**5**) showed the presence of the molecular ion at *m/e* = 326.

Alkaline hydrolysis of the amino ester (**5**) afforded the corresponding amino acid which precipitated from the solution on careful titration to the isoelectric point first with conc HCL and then dil HCL maintaining a minimum volume of aqueous solution.

The crucial reaction of the synthetic sequence was next attempted. It is known that benzylic C—N bonds of tertiary amines can be attacked with acetic anhydride by the attack of acetate anion on the carbon, cleavage of C—N bond and acylation of the

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†A portion of this work has been published as a preliminary communication.[14]

nitrogen.[2-6] Similar cleavages on the hexahydroindolopyrrocoline system had not been attempted before. Model reactions carried out by Harley-Mason *et al.* showed that it was possible to cleave the hexahydroindolopyrrocoline system with cold acetic anhydride in high yields.[7] Dolby had demonstrated an interesting variation of this reaction on dihydrocorynantheine derivatives involving attack of the acetate anion, cleavage of C—N bond and concurrent acylation of the nitrogen with a suitable acylating side chain.[8] It was therefore envisaged that when the amino acid was treated with acetic anhydride, it would afford the desired lactam.

When the amino acid was heated with acetic anhydride, tlc showed complete conversion to two faster running spots. The product (**6**) crystallised from ethyl acetate (m.pt. 227°) in 60% yield. The presence of ester and lactam CO functions was indicated by bands at 1733 cm^{-1} in the IR spectrum. The elemental analysis agreed with the formulation $C_{21}H_{26}N_2O_2$ for the compound. The base peak in the mass spectrum was at $m/e = 354$ which was also the molecular ion. The presence of the acetoxy function was also shown by the mass spectrum which showed the expected loss of acetic acid from the molecule. The C-16 proton in the acetoxy lactam appeared as a doublet* centred at 86.14 with a coupling constant of 10 c/s.

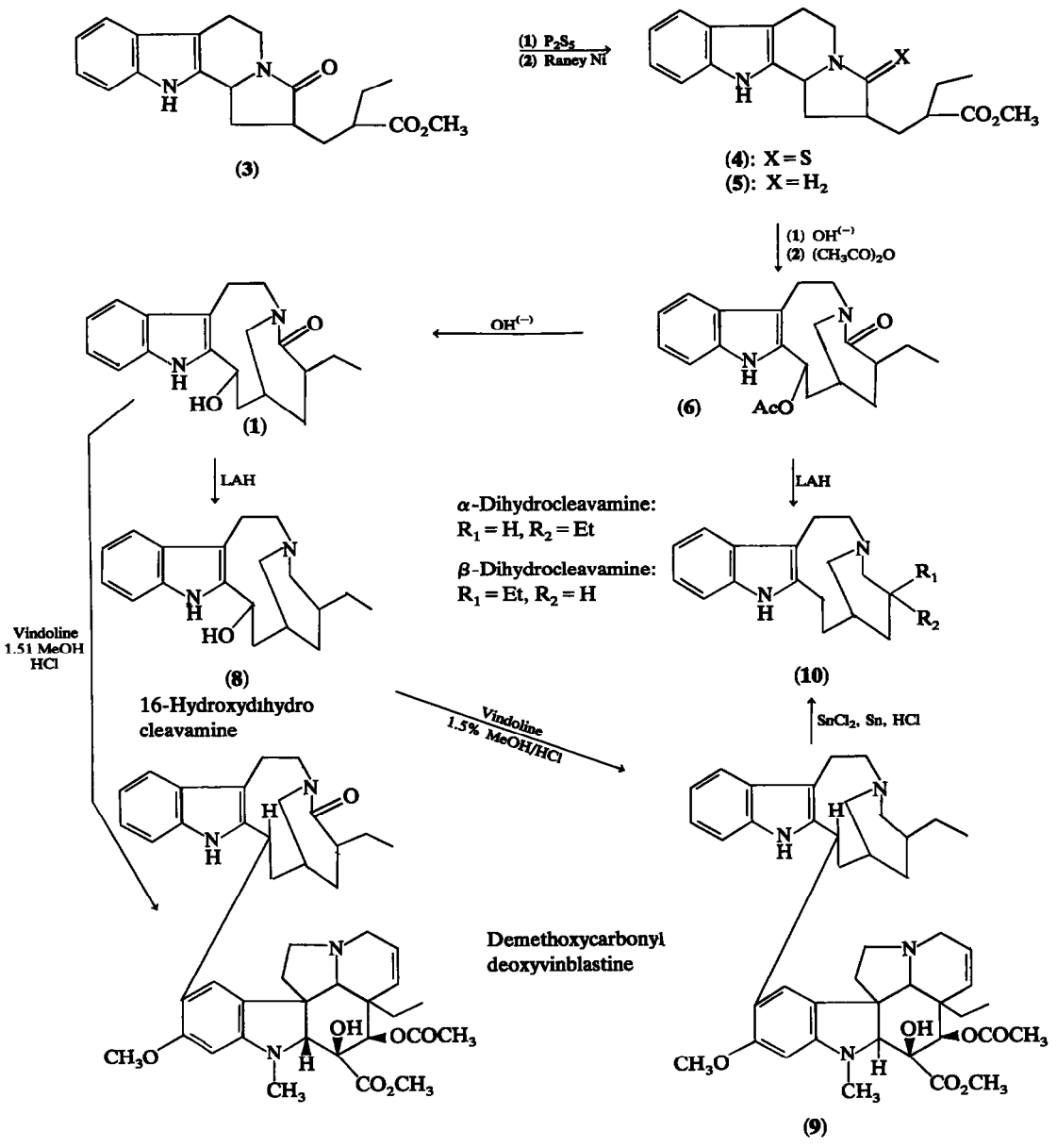
Hydrolysis of the acetoxy lactam with aqueous ethanolic sodium hydroxide afforded the corresponding hydroxy compound (**7**) in quantitative yields. The IR spectrum of this compound showed the presence of the lactam CO but the absence of the ester grouping. On recrystallisation from acetone the compound was obtained as colourless needles m.pt. 242°. The identity of the compound was confirmed from its mass spectrum which showed the molecular ion at $m/e = 312$ and a predictable fragmentation pattern. Confirmation of the location of the OH group in the hydroxy lactam (**7**) came by oxidising it to the corresponding keto compound either with manganese dioxide or with t-butyl hypochlorite, the reaction with the latter reagent proceeding in quantitative yields. The product obtained was readily crystallisable and possessed a UV spectrum characteristic of 2-acyl indoles. This unambiguously established the location of the hydroxyl group in **7** and hence of the acetoxy group in **6**. The oxidation with t-butyl hypochlorite probably proceeds via the intermediate formation of the corresponding chloroindolenine which then rapidly loses hydrogen chloride to afford the 2-acyl indole. The reaction may be of general use and possess advantages over the conventional oxidation with manganese dioxide for transformation of the 16-OH to compounds to the corresponding 2-acyl indoles.

Reduction of the ketolactam with sodium borohydride afforded four spots on tlc which were found to be the four possible diastereoisomers of **7**. The two major spots were found to be identical to the two diastereoisomers obtained on hydrolysis of **6**. This showed that the attack of acetate occurs stereospecifically in the formation of **6**. The faster and slower running diastereoisomers for the acetoxylactam (**6**) were isolated by preparative tlc. The slower running compound on hydrogenolysis with LAH afforded only α-dihydrocleavamine, thus establishing that the et group in the slower running epimer was in the α-configuration i.e. in a *trans* disposition with respect to the quinuclidine nitrogen. Similarly the faster running racemate of the acetoxylactam was shown to possess the β-configuration at C-20. This faster running racemate on hydrolysis yielded only the faster running of the two hydroxylactams (**7**) indicating that a similar situation prevailed in the hydroxylic compounds.

LAH reduction of the hydroxylactam afforded a new compound in 90% yield as a white powder. The IR spectrum of the reduction product showed the absence of the lactam CO and the presence of an OH function. Low and high resolution mass spectroscopy confirmed the expected structure **8** for 16-hydroxydihydrocleavamine.

From the available evidence in literature,[10-12] it seemed likely that (±)-16-hydroxydihydrocleavamine (**8**) would react with vindoline under acid conditions to afford the desired binary molecule. The synthetic (±)-16-hydroxydihydrocleavamine (**8**) was therefore added to a solution of an excess of vindoline in 1% anhydrous methanolic hydrogen chloride. The reaction was followed by tlc and it was observed that at room temperature, the starting material was very quickly converted to two new slower-running compounds. The reaction was conducted in the absence of light and oxygen because of the known tendency of these compounds to undergo photodegradation readily. Tlc showed that some vindoline was still present, and besides a base spot, two new compounds had been formed. Since these compounds tended to decompose if column chromatography was used for their purification, it was found most convenient to obtain them by preparative tlc. A low resolution mass spectrum on the mixture of the two compounds isolated showed the molecular ion at $m/e = 736$ in agreement with the formulation $C_{44}H_{56}N_4O_6$ for these binary compounds. Separation of the two compounds by preparative tlc and examination of their individual mass spectra established them to be chemically identical. The general cleavage fragmentation pattern was also comparable with that of VLB. A high resolution mass spectrum on the molecular ion confirmed that the molecular formula was $C_{44}H_{56}N_4O_6$. The UV spectrum provided additional confirmatory evidence for the identity of the binary molecule (**9**) with maxima at 215, 262, 285, and 293 millimicrons in close agreement with the published values for the UV spectrum of VLB.

* The reason for the presence of a doublet rather than a multiplet was apparent from an examination of Dreiding models. One of the two protons adjacent to the C-16 proton can be situated at an angle of 90° to the latter so that it would not show any coupling. Such a conformation in the acetoxy lactam would also have the least non-bonded interactions. The fact that the corresponding C-16 proton in the indole moiety of desacetyl VLB hydrazide also appears as a doublet with the same coupling constant (10 c.p.s.) lends support to this suggestion.[9]



Since the yields obtained in the formation of these binary molecules were relatively low, it was decided to investigate this reaction more carefully. Blank experiments carried out by treating 16-hydroxydihydrocleavamine alone with 1% methanolic hydrogen chloride showed on tlc that this compound was rapidly converted to a base spot and some slower running spots on the plate. One can evisage a self-dimerisation of 16-hydroxydihydrocleavamine in analogy with the self-dimerisation of 1-hydroxy-1,2,3,4-tetrahydrocarbazole. Close and consistent examination of tlc plates after these reactions indicated that by-products were being formed in only minor quantities and by far the major portion of the reaction product was the desired binary compound. Since no 16-hydroxydihydrocleavamine was left at the end of the reaction, the low yields in these reactions appears primarily to be due to unsatisfactory recovery from the plates rather than formation of the binary compound in low yields.

Initially binary products were obtained as gums and provide difficult to crystallise. However, when the two spots were separated by preparative tlc it was found possible to crystallise the slower of the two spots from ethanol and a correct elemental analysis was obtained after several recrystallisations from ethanol.

The NMR spectrum unambiguously established that C-16 of the tetracyclic indole moiety was attached to vindoline. Instead of the typical 1,2,4-pattern (δ 6.9, δ 6.3, and δ 6.08) present in vindoline, only two protons in a 1,4-relationship were found to be present. These were located at δ 6.27 and δ 6.63. Neuss had found a similar situation in the NMR spectra of the naturally occurring binary molecules such as VLB, VCR etc and in fact it was this NMR evidence that had established the posi-

tion of linkage of C-16 of the indole moiety in the original structural determination of VLB and VCR.[13] The NMR of the binary molecule established that the attack at C-16 was stereospecific, the C-16 H resonating as a uniform doublet at δ 4.5 and integrating for a full proton.* The C-16 H of the binary molecule with the opposite configuration at C-16 is known to resonate further upfield.[9] The stereospecificity of the nucleophilic attack lowers the number of possible binary compounds to four instead of eight. It was suspected that each band on tlc contained two diastereoisomers. In order to check this, the faster running spot was isolated by preparative tlc and reductively cleaved by refluxing with tin, stannous chloride and concentrated hydrochloric acid. Examination of the cleavage products by tlc showed that both α- and β-dihydrocleavamines were being formed. This indicated that two diastereoisomers were actually present in this single spot, corresponding to the two possible epimers at C-20. In order to eliminate the stereochemical complexities, it was decided to prepare demethoxycarbonyldeoxy-VLB bearing the Et group at C-20 in the β-configuration only. As mentioned earlier, by careful chromatography it was found possible to separate the two acetoxylactams epimeric at C-20 and their relative configuration was established by hydrogenolysis to α- and β-dihydrocleavamines.

Since the faster running epimeric acetoxylactam corresponded to the ethyl group in the β-configuration, it was decided to use this in the synthesis of the binary molecule. A repetition of the same procedure then afforded demethoxycarbonyldeoxy-VLB which ran as two spots, which could be reductively cleaved to the optically active β-dihydrocleavamines. Similarly it was possible to separate the optically active α-dihydrocleavamine by starting from the slower running acetoxylactam (which bears the ethyl group in the α-configuration) and repeating the same sequence.

A high yield synthesis of vinblastine by Polonovski reaction of dihydro-catharanthine-N-oxide with vindoline and $Th(OAc)_3$ oxidation/reduction of the resulting binary enamine was described by one of us (A. R.) in 1978[18] and has later also been reported by Potier.[19] Osmylation-reduction of the same binary enamine affords vinrosidine.[18,19]

EXPERIMENTAL.

IR spectra were determined on a Unicam SP. 200 or Perkin-Elmer 137 spectrophotometer. UV spectra were recorded on Cary model 145 M-50 spectrophotometer or on a Perkin-Elmer Model 137 UV instrument. UV spectra were measured in 95% etol. NMR spectra were recorded on a Varian 100 Mc/s instrument using tetramethylsilane as an internal standard. Mass spectra were recorded on an AEI MS 9 spectrometer. All mass spectra were determined after direct insertion of the sample. Column chromatography was generally carried out using Woelm activity III neutral alumina. Silicic acid was also used. Tlc was generally carried out on fluorescent silica gel GF-254 plates. Preparative tlc was carried out with coatings of 0.6 mm. or 1.0 mm thickness of the same material. Substances were separated in a dark room under UV light. Mg ps were determined microscopically with a Kofler block. All mg ps are uncorrected. Gas–liquid chromatography was carried out on a Perkin-Elmer F-II gas chromatograph or F+M model 720 dual column gas chromatograph.

Preparation of 2 (2'-*methoxycarbonyl butane*)-3 *thio*-1,2,3,5,6, 11*b*-*hexahydro*-11-H-*indolo*-I (3,2-g)-*pyrrocoline* (**4**). The lactam **3** (1.34 g, 0.00395 mole) was dissolved in anhyd xylene (140 ml). The soln was magnetically stirred and heated to 105° till all the ester dissolved. Then P_2S_5 (0.91 g, 0.0041 mole) was added and the soln left stirring under N_2 at 105° for 1 hr. Tlc showed the complete conversion of the ester **3** to a new compound at the end of this period. The hot soln was decanted off from the solid, extracted with water and the organic layer dried Na_2SO_4 and evaporated to a pale yellow gum (1.1 g, 79% yield). A quick filtration of a soln of the gum in EtOAc through activity III alumina (10 gm) removed the colour and provided colourless plates, m.pt 156°. IR spectrum: ν max 3340 cm^{-1} (–NH), 1720 cm^{-1} (ester C=O), 1500 cm^{-1} (amide II band), 1110 cm^{-1} (C=S of thiolactam); UV spectrum: λ max 223, 274 nm, ε max 37200, 26700, λ min 240 nm, ε min 4550; mass spectrum: *m/e* 356 (100%), 325 (18%), 323 (37%), 296 (5%), 243 (10%), 242 (72%), 209 (15%), 182 (12%), 170 (29%), 169 (27%), 168 (30%), 167 (21%), 156 (23%), 155 (14%), 154 (14%), 148 (8%), 144 (15%), 143 (15%); Analysis: Calc: C, 67.4, H, 6.7, N, 8.0%; Observed: C, 67.0, H, 6.8, N, 7.8%).

Preparation of 2(2'-*carbomethoxy butane*)-1,2,3,5,6, 11*b*-*hexahydro*-11-H-*indolo*-(3,2 – g)-*pyrrocoline* (**5**). The ester **4** (3.0 g, 0.0084 mole) was dissolved in 80% EtOH. Freshly prepared W-6 Raney Ni (9.10 g), was added to the magnetically stirred solon, care being taken not to allow any of the Raney Ni to stick to the sides of the flask. The reaction was followed by tlc and complete conversion of 4 to a slower moving spot was observed within 15 min. The solon was quickly filtered through a 4 inch column of "supercel" to remove any colloidal suspension of Ni. The colourless filtrate was evaporated on the rotary evaporator to a gum (2.4 g, yield = 81%).

A crystalline methiodide of the ester was obtained from EtOAc (m.pt. = 188°); IR spectrum: ν max 1722 cm^{-1} (C=O ester); UV spectrum (*of methiodide*): λ max 224, 274, 279, 290, ε max 57800, 7810, 7310, 5720, λ min 244, 276, 286, ε min 2320, 7140, 4460; analysis (*of methiodide*); cal: C, 53.8, H, 6.2, N, 6.0% observed: C, 53.7, H, 6.2, N, 5.9%; mass spectrum: *m/e* = 326 (M^+,43%) 325 (100%), 324 (9%), 311 (4%), 294 (8%), 209 (5%), 184 (5%).

Preparation of 2(2'-*carboxy butane*)-1, 2, 3, 5, 6, 11b-*hexahydro*-11-*H*-*indolo*-(3, 2,-g)-*pyrrocoline*. The ester **5** (2.3 g, 0.00705 mole) was dissolved in 70% aqueous MeOH (175 ml) containing KOH (3.0 g). The solon was refluxed for 10 min, cooled and evaporated to a viscous yellow liquid. Acidification, initially with conc HCl and then, when the isotropic point was approached, dropwise with dil HCl resulted in the precipitation of a yellowish solid (2.0 g, 90% yield). Purification was carried out by trituration of the yellowish solid with acetone when colourless crystals were left behind. Recrystallisation from acetone afforded pure crystals, m.pt. 219–223°. IR spectrum: ν max 1710 cm^{-1} (unionised–COOH), 1540 cm^{-1} (asymmetric–COOH); UV spectrum; λ max 223, 274, 279, 289 nm, ε max 32600, 7030, 6960, 5560, λ min 224, 276, 287 nm, ε min 2280, 6760, 4960, λ inflection 281 nm, ε inflection 6900, (found: calc: C, 73.1, H, 7.7, N, 8.9%) C, 73.3, H, 8.0, N, 8.9%), mass spectrum: *m/e* = 312 (M^+, 90%), 311 ($M^+ - 1$, 100%), 292 (4%), 268 (9%), 267 (12%), 211 (10%), 209 (13%), 208 (7%), 207 (5%),

*The subsequent work in this field by one of us (A. R.)[15–17] leads us to suspect that the binary molecule obtained has the unnatural configuration at C-16.

185 (7%), 184 (44%), 169 (8%), 168 (11%), 167 (19%), 156 (13%), 144 (5%), 143 (4%).

Preparation of 16-*acetoxy*-21-*oxo-dihydrocleavamine* (**6**). To the amino acid (1.4 g, 0.00448 mole) was added freshly distilled analar Ac_2O (25 ml). The solon was stirred magnetically and heated in an atmosphere of N_2 at 85–88° for 6 hr on an oil bath; tlc then showed the complete conversion of the polar starting material to two faster running products. The solon was allowed to cool and evaporated on a rotary evaporator to a brownish gum. This was dissolved in EtOAc (25 ml). and extracted twice with 25 ml portions of distilled water. The EtOAc layer was separated, dried ($MgSO_4$) and evaporated to a brownish gum which slowly crystallised on standing to afford colourless crystals, m.p. 227°. (0.960 g, yield = 60%).

Alternative preparations involving the use of Ac_2O with NaOAc gave lower yields. IR spectrum: ν max 1732 cm^{-1} (C=O of acetoxy group), 1635 cm^{-1} (C=O of lactam); UV spectrum: λ max 224, 277, 285, 294 nm, ε max 41300, 8560, 9000, 7190, λ min 246, 280, 291 nm, ε min 2690, 8460, 7030, λ inflection 320 nm, ε inflection 464; analysis: calculated: C = 71.2, H = 7.3, N = 7.9%, observed: 71.6, H = 7.4, N = 7.7%; mass spectrum: *m/e* = 354 (*M*$^+$, *100%*), 340 (10%), 325 (6%), 311 (9%), 297 (12%), 296 (54%), 295 (35%), 294 (65%), 293 (13%), 279 (11%), 266 (12%), 265 (16%), 251 (4%), 238 (4%), 237 (9%), 236 (12%), 225 (5%), 224 (12%), 223 (10%), 222 (6%), 210 (13%), 209 (25%), 208 (12%), 207 (7%), 206 (5%), 200 (11%), 197 (6%), 196 (9%), 195 (10%), 194 (13%), 187 (15%), 186 (5%), 185 (9%), 184 (15%), 183 (11%), 182 (21%), 181 (10%), 180 (21%), 174 (11%), 173 (7%), 172 (19%), 171 (18%), 170 (66%), 169 (42%), 168 (45%), 167 (24%), 159 (23%), 158 (55%), 157 (31%), 156 (57%), 155 (15%), 154 (17%), 144 (60%), 143 (42%), 138 (30%), 130 (30%), NMR spectrum: δ 6.9–7.7 multiplet (4H, aromatic protons), δ 6.15, doublet (10 c/s 1H, C-16 proton). The pure crystals ran as two spots on tlc in several solvent systems.

Preparation of 16-*hydroxy*-21*oxo*-*dihydrocleavamine* (**7**). Compound **6** (0.350 g, 0.00099 mole) was dissolved in boiling EtOH (150 ml). It was then hydrolysed at room temp in 80% EtOH (250 ml) containing NaOH (0.75 g). The reaction was following by tlc. After 4 hr, complete conversion of **6** to another compound was observed. The solon was evaporated on a rotary evaporator to remove all the EtOH. The viscous aqueous layer was then thoroughly extracted with $CHCl_3$. The $CHCl_3$ layer was separated and dried (Na_2SO_4). Evaporation of the $CHCl_3$ solon afforded a white solid (0.310 g, yield = 99%). This was crystallised as colourless needles, m.p. 242°, from acetone. The crystals ran as two spots on tlc. IR spectrum: ν max 1630 cm^{-1} (lactam C=O), 3250 cm^{-1} (indole NH); UV spectrum: λ max 225, 285, 294 nm, ε max 42700, 8600, 7390, λ min 247, 291 nm, ε min 1610, 6780, λ shoulder 278 nm, ε shoulder 7930; Found: C, 73.20, H, 7.82, N, 8.97; Calc: C, 73.07, H, 7.69, N, =8.97%), mass spectrum: *m/e* = 312 (*M*$^+$, 100%), 311 (16%), 310 (10%), 294 (5%), 282 (6%), 280 (4%), 188 (10%), 187 (54%), 186 (9%), 185 (19%), 184 (12%), 174 (15%), 173 (16%), 172 (43%), 171 (6%), 170 (14%), 169 (8%), 168 (12%), 167 (6%), 159 (20%), 158 (22%), 157 (11%), 156 (16%), 155 (5%), 154 (12%), 144 (62%), 143 (58%), 142 (13%), 130 (28%), 126 (14%), 125 (16%).

Preparation of (+)-16-*hydroxydihydrocleavamine* (**8**). Compound **7** (0.056 g, 0.000165 mole) was dissolved in anhyd THF (10 ml). Fresh LAH (0.042 g) was quickly added and the solon refluxed in the dark for 1 hr. The solon was cooled and the excess LAH was carefully destroyed with a few drops of water. The slurry was then evaporated under vacuum to a white powder. This powder was scraped out, placed in a Soxhlet thimble and extracted with benzene in a Soxhlet apparatus for 18 hr in the absence of light. The benzene solon was evaporated to a gum which solidified from aqueous acetone to afford a white powder (0.050 g, yield = 99%). The compound was found to be very susceptible to auto-oxidation. The compound was found to run as two spots on tlc (Rf value 0.85 and 0.9 in 92% $CHCl_3$: 8% MeOH). IR spectrum: ν max 3595 cm^{-1} (O—H); mass spectrum *m/e* = 298 (M$^+$, 44%), 297 (3%), 296 (4%), 281 (1%), 282 (2%), 274 (2%), 254 (2%), 234 (2%), 215 (3%), 195 (2%), 185 (6%), 184 (2%), 180 (2%), 174 (7%), 173 (60%), 172 (4%), 170 (4%), 169 (6%), 158 (9%), 149 (10%), 145 (4%), 144 (21%), 143 (8%), 140 (6%), 139 (9%), 138 (100%), 137 (10%), 130 (13%), 125 (65%), 125 (17%), 124 (35%); NMR spectrum ($CDCl_3$): δ 6.9–7.6 multiplet (4H, aromatic protons), δ 5.76, doublet (10 c/s, 1H, C-16 proton).

Hydrogenolysis of 16-*acetoxy*-21-*oxo-dihydrocleavamine* (**6**) *to* α- and β-dihydrocleavamines (**10**). The mixture of the two epimeric lactams **6** (0.005 g, two spots on tlc) was dissolved in anhyd THF and refluxed with a hundred molar excess of LAH for 4 hr while the solon was magnetically stirred. The solon was allowed to cool and the excess of LAH destroyed by careful addition of drops of water. The aqueous solon was then extracted into CH_2Cl_2, the CH_2Cl_2 extracts dried (Na_2SO_4) and evaporated to afford a colourless glass (0.004 g). Tlc and spectroscopic comparison showed that the two products which had been formed during the hydrogenolysis experiment were identical with authentic samples of α- and β-dihydrocleavamines.

Oxidation 16-*hydroxy*-21-*oxo-dihydrocleavamine* (**7**) *with manganese dioxide to* 16-*keto*-21-*oxo-dihydrocleavamine*. Compound **7** (0.02 g, 0.00064 mole) was dissolved in a $CHCl_3$: CH_2Cl_2 mixture (2:1) The solon was magnetically stirred and active MnO_2 (0.12 g) was added. The solon was allowed to stand at room temp for 30 hrs. Tlc showed the complete conversion of **7** to two faster running spots (Rf. value 0.95 and 0.90 in 92% $CHCl_3$). The solon was filtered twice to completely remove any MnO_2 and evaporated to afford a crystalline solid (m.p. = 228–232°) IR spectrum: ν max 1630 cm^{-1} (lactam C=O), 1655 cm^{-1} (C—O of keto group), 3320 cm^{-1} (indole N—H); UV spectrum: λ max 210, 238, 319, nm λ min 226, 270, nm λ inflection 350 nm; mass spectrum: *m/e* = 311 (19%), 310 (*80%*, *M*$^+$), 283, (9%), 282 (40%), 186 (22%), 185 (100%), 184 (30%), 183 (8%), 173 (7%), 172 (10%), 171 (7%), 170 (10%), 169 (11%), 168 (22%), 166 (10%), 158 (14%), 157 (20%), 156 (11%), 149 (9%), 144 (28%), 143 (50%), 141 (19%), 130 (19%), 129 (19%), 128 (10%), 127 (7%), 125 (24%), 124 (9%), 115 (9%).

Reduction of 16-*keto*-21-*oxo-dihydrocleavamine to* 16-*hydroxy*-21-*oxo-dihydrocleavamine* (**7**). 16-Keto-21-oxo-dihydrocleavamine (0.003 g) was dissolved in 1:2-dimethoxyethane. The solon was magnetically stirred and fresh $NaBH_4$ (0.003 g) was added. Tlc after 90 min showed the complete conversion of the starting material to four new products. Two of these had the same Rf value as an authentic sample of 16-hydroxy-21-oxo-dihydrocleavamine prepared previously. The other two had a lesser Rf value. Mass spectrometric examination showed all four to be the various possible diastereoisomers of **7**.

The partial synthesis of "demethoxycarbonyldeoxy-vinblastine" (**9**) Compound **8** (0.02 g, 0.000067 mole) (two spots on tlc epimeric at C-20) was dissolved in anhyd MeOH (2.0 ml). Vindoline (0.08 g, 0.00018 mole) was separately dissolved in 1.5% solon of anhyd methanolic HCl (2 ml). The methanolic solon of 16-hydroxy-dihydrocleavamine was then added dropwise to the solon of vindoline. The solon was magnetically stirred in the absence of light during this addition and allowed to stand for several hr in an atmosphere of N_2 at room temp. Tlc

showed immediate conversion of 16-hydroxydihydrocleavamine to mainly two slower running spots. Some excess vindoline was also present at the end of the reaction. The methanolic solon was evaporated off and the white powder of the hydrochlorides of the bases obtained dissolved in water. The aqueous solon was basified with Na_2CO_3 when a white ppt appeared. This was dissolved in EtOAc and the aqueous solon extracted thrice with EtOAc (50 ml). The EtOAc extracts were dried (Na_2SO_4) and evaporated to a colourless gum. Careful tlc (91% $CHCl_3$: 9% MeOH) of the gum showed that no 16-hydroxydihydrocleavamine was present but two major slower running products. (Rf value 0.7 and 0.75 respectively) had been formed. Beside these two spots there was a faster running spot (Rf value 0.95) corresponding to unreacted vindoline and two slow running faint spots (Rf value 0.2 and 0.25) as found for the two faint spots in the control reaction. The latter appear therefore to be self-dimerisation or polymerisation products of (±)-16-hydroxydihydrocleavamine.

The two major products formed during this reaction were separated by preparative tlc on silica gel GF-253 plates from other compounds present. The silica gel powder was scraped off the plates and stirred with anhyd MeOH for several hr in the dark. Filtration of the methanolic solon and evaporation of the filtrate afforded a colourless gum which still contained some silica gel. The gum was redissolved in benzene and the benzene solon filtered and evaporated to afford a colourless glass (0.014 g) which ran as only two spots (Rf 0.70 and 0.75 in 91% $CHCl_3$: 9% MeOH).

The two compounds thus obtained could be easily separated by careful preparative tlc and were demonstrated by mass spectrometry to be chemically identical. The slower running of the two spots was crystallised from EtOH as colourless needles, m.p. 162°–168°. UV spectrum λ max 312, 262, 285, 293 nm λ min 243, 280, 290, 221, 320 nm (ultra-violet spectrum very similar to that of VLB). NMR spectrum: δ 6.27, δ 6.63, two singlets, (2H aromatic protons of vindoline moiety) δ 6.8–7.6 multiplet (4H, aromatic protons of indole moiety). δ 4.5 doublet, 10 c.p.s (1H, C-16 proton of indole moiety), δ 3.9 singlet (3H, methoxyl protons of vindoline moiety), δ 3.72 singlet (3H, ester protons of vindoline moiety), δ 2.68 singlet (3H, N-methyl protons); mass spectrum: *m/e* = 736 (*M*$^+$, *71%*), 735 (33%), 734 (40%), 733 (13%), 732 (16%), 131 (4%), 730 (8%), 723 (3%), 722 (4%), 721 (3%), 719 (3%), 707 (7%), 694 (4%), 693 (4%), 692 (4%), 691 (4%), 678 (5%), 677 (8%), 676 (7%), 675 (7%), 674 (4%), 660 (5%), 659 (4%), 612 (4%), 611 (5%), 610 (4%), 609 (6%), 606 (9%), 599 (7%), 598 (15%), 577 (8%), 576 (25%), 575 (9%), 574 (5%), 564 (3%), 555 (4%), 539 (4%), 495 (8%), 470 (7%), 469 (15%), 468 (9%), 467 (13%), 466 (11%), 452 (6%), 451 (5%), 450 (5%), 439 (7%), 438 (7%), 386 (7%), 383 (5%), 368 (10%), 367 (7%), 351 (4%), 341 (5%), 340 (8%); 339 (11%), 325 (7%), 311 (7%), 293 (11%), 282 (26%), 281 (10%), 280 (12%), 279 (10%), 278 (32%), 277 (14%), 276 (7%), 138 (78%), 135 (100%), 124 (32%), 122 (50%); high resolution mass spectrum: on molecular ion, *m/e* = 736: calc: 736. 420, found: 736.421132; Found C, 71.87, H, 7.60, N, 7.2%, Calc C, 71.74, H, 7.60, N, 7.60).

Preparation of 20-β-*demethoxycarbonyldeoxyvinblastine* (**9**). Compound **6** was carefully chromatographed over as Activity I alumina column and the faster running diastereoisomer isolated (0.019 g). (This was shown to possess the Et group in a β-configuration by a hydrogenolysis experiment described previously). Alkaline hydrolysis as before in ethanolic KOH afforded 16-hydroxy-21-oxo-β-dihydrocleavamine (0.016 gm) (faster running diastereoisomer). LAH reduction in anhyd THF by the same procedure as described earlier afforded 16-hydroxy-β-dihydrocleavamine (0.012 g) (faster running diastereoisomer). Reaction with vinoline in methanolic HCL afforded a product which ran as two spots as before on tlc isolation by preparative tlc afforded a colourless glass (20 β-demethoxycarbonyldeoxyvinblastine) which crystallised from EtOH and was shown by mass spectrometry, UV tlc etc to be chemically identical with demethoxycarbonyldeoxyvinblastine prepared previously.

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