Compound (Ia): Yield 45%; m.p. 98–100 °C; $[\alpha]_D^{20} = -18°$ (c 1 in $CHCl_3$); I.R. ν_{max} 3520–3400, 1720, 1640, 1445 cm^{-1} and no evidence of amide II band; NMR δ ppm 4.10 (1H, q, J = 6.5 Hz, Ala $C_\alpha H$), 4.84 (1H, 4 lines, X part of an ABX, Phe $C_\alpha H$), 3.7 (1H, m, Pro $C_\alpha H$ superimposed on Pro $C_\delta H_2$ multiplets), 4.90 (1H, bs, OH); MS *m/e* 399 (M^+, 19%), 381 ($M-H_2O$, 2%), 125[6] (3%), 70 (base peak). Hydrazinolysis[7] of (Ia) gave allyloxycarbonyl-Ala-$NHNH_2$ and cyclo (-Phe-Pro-).

Compound (IIa): Yield 15%; glassy oil; $[\alpha]_D^{20} = +114°$ (c 1 in $CHCl_3$); I.R. ν_{max} 3430 (carbamate NH), 1710, 1660, 1495 cm^{-1} (amide II); NMR δ ppm 5.38 (1H, q, J = 6.5 Hz Ala $C_\alpha H$), 2.35 (1H, m, Pro $C_\alpha H$), 5.20 (1H, m, Phe $C_\alpha H$), 5.45 (1H, unresolved, NH); MS *m/e* 399 (M^+, 10%), 381 ($M-H_2O$, less than 0.1%), 125[6] (26%), 128 (base peak). Hydrazinolysis[7] of (IIa) gave allyloxycarbonyl-Ala-$NHNH_2$ and cyclo(-Phe-D-Pro-).

It is known that N-hydroxyacyl-lactams and N-hydroxyacyl-diketopiperazines can give rise to oxacyclols[8–12]. In view of the probable existence of N-acyldiketopiperazines as reaction intermediates in the formation of cyclols from linear acyl-tripeptides *p*-nitrophenylesters, and because of the easy epimerization of the N-acyldiketopiperazines containing proline in polar medium[10], it seemed interesting to examine the reactivity of a cyclol in mild alkaline aqueous buffer. Azacyclol (Ib)[1] was then allowed to stand 1.5 h at room temperature in the buffer already cited[5]. Removal of dioxane and usual fractionation gave Z-Ala-Phe-D-Pro and Z-Ala in acidic fraction. From the neutral fraction, 4 main components could be isolated by TLC. Composition of the neutral fraction was as follows: starting azacyclol (Ib) (56%), cyclo(-Phe-D-Pro-) (17%), azacyclol (III) (10%) and acyl-diketopiperazine (IIb) (17%). Structure (IIb) and (III) were assigned on the basis of chemical and spectral properties.

Compound (IIb): Colourless foam; $[\alpha]_D^{20} = +82°$ (c 2 in $CHCl_3$); I.R. ν_{max} 3430, 1710, 1655, 1495 cm^{-1}; NMR δ ppm 5.47 (1H, q, J = 7.0 Hz, Ala $C_\alpha H$), 2.40 (1H, m, Pro $C_\alpha H$), 5.27 (1H, t, J = 5.0 Hz, Phe $C_\alpha H$), 5.78 (1H, d, J = 8.5 Hz, NH); MS *m/e* 449 (M^+, 4.5%), 431 ($M-H_2O$, 0.4%), 125[6] (32%), 91 (base peak). Hydrazinolysis[7] of (IIb) gave cyclo(-Phe-D-Pro-) and Z-Ala-$NHNH_2$. Compound (IIb) could be synthesized in high yield by treating Z-Ala-Phe-Pro with excess Ac_2O-AcONa at 100 °C for 1 h.

Compound (III): Colourless foam; soluble in 1 *N* NaOH from which can be reprecipitated on acidification. $[\alpha]_D^{20} = +71°$ (c 1 in EtOH); I.R. ν_{max} 3500–3300, 1715, 1645, 1440 cm^{-1}; NMR δ ppm 4.30 (1H, q, J = 7.0 Hz, Ala $C_\alpha H$), 4.40 (1H, m, Pro $C_\alpha H$), 4.15 (1H, unresolved m, X part of an ABX, Phe $C_\alpha H$), 6.30 (1H, bs, OH); MS *m/e* 449 (M^+, 30%), 431 ($M-H_2O$, 2.5%), 125[6] (17%), 91 (base peak). Hydrazinolysis[7] of (III) gave cyclo(-Phe-D-Pro-) and Z-Ala-$NHNH_2$.

When the same treatment with the alkaline buffer was applied to N-acyldiketopiperazine (IIb), a reaction mixture containing the same components as for azacyclol (Ib) was obtained. In this case the composition of the neutral fraction was as follows: starting material (IIb) (35%), cyclo(-Phe-D-Pro-) (40%), azacyclol (Ib) (8%), azacyclol (III) (18%).

The above results seem to indicate that in the mild alkaline medium an equilibrium can be established between acyl-*trans*-diketopiperazine (IIb) and its *cis*-isomer. Each isomer can in turn equilibrate with the corresponding azacyclol. The *cis* -isomer of (IIb) could not be detected, and this fact can be ascribed to the already known instability of these *cis*-isomers[10] and to a higher tendency to isomerize into the corresponding azacyclol (Ib). The different reactivity between acyl-*trans*-diketopiperazine (IIb) and the corresponding *cis*-isomer can be reasonably related to the already known different conformations between DL and LL isomers of cyclic dipeptides[13,14]. Such different conformation should influence the reactivity of the amide carbonyl; in the *cis*-isomer the amide bond is in fact forced into a slightly non-planar arrangement. When the synthesis of the *cis*-isomer of (IIb) was attempted by reacting Z-Ala-Cl with the N-trimethylsilyl derivative of cyclo(-Phe-Pro-), only azacyclol (Ib) was obtained.

The base catalyzed interconversion, observed by us in the case of the described azacyclols, was not found in the case of oxacyclols containing the same diketopiperazine system. Such different behaviour could possibly derive either from a higher instability of the corresponding hydroxyacyl-diketopiperazines or from a greater stability of the oxacyclols.

Riassunto. È stata studiata la reattività in ambiente acquoso blandamente alcalino dei cicloli tripeptidici. Si è messo in evidenza un equilibrio tra i sistemi azaciclolici attraverso le corrispondenti acil-alanil-dichetopiperazine.

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[6] Peak at *m/e* 125 is the base peak in the MS of cyclo(-Phe-Pro-).

[7] Hydrazine hydrate (2 moles) was added to 1% methanolic solution of the compound (1 mole). The mixture was left 18 h at room temperature. By this treatment N-propionyl-cyclo(-Phe-Pro-) gave cyclo(-Phe-Pro-). For this procedure see: A. HOFMANN, H. OTT, R. GRIOT, P. A. STADLER and R. J. FREY, Helv. chim. Acta *46*, 2306 (1963).

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Vinca Alkaloids XXXV.[1] Desacetoxyvinblastine a New Minor Alkaloid from *Vinca rosea* L. (*Catharanthus roseus* G. Don)

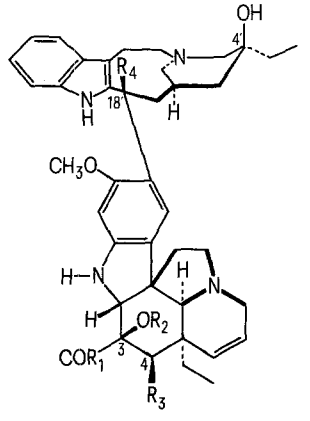
In the process of purifying larger quantities of VLB[2] (vincaleukoblastine (I)), we have noticed the presence of a new dimeric indole-indoline alkaloid. Physical and chemical data clearly indicated that the new compound is desacetoxyvinblastine (II).

The UV and IR spectra of VLB and desacetoxy VLB are quite similar. The nature of the difference between the 2 alkaloids is immediately apparent from the NMR and mass spectral data. Thus, the signal of the acetyl methyl of VLB (s, δ = 2.10 ppm)[3] is missing in the NMR-

Table I. Chemical shifts of acetyl methyl signals (ppm)

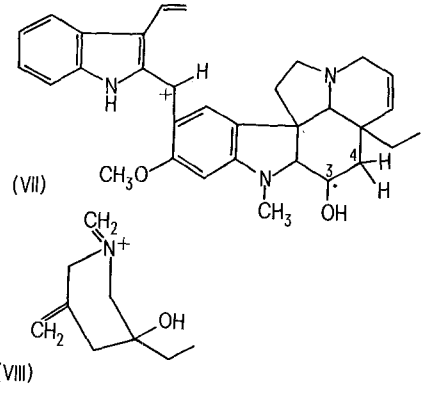
	VLB (I)	VLB acetate (III)	Desacetoxy VLB (II)	Desacetoxy-VLB-acetate (IV)
C-3-acetyl	—	1.96	—	1.90
C-4-acetyl	2.10	2.08	—	—

spectrum of desacetoxy VLB. Chemical shifts in the NMR-spectra of VLB-diacetate and desacetoxy VLB-acetate, both prepared by acetylation of the base using Ac_2O in Py, are shown in Table I. It should be noted that under these conditions the tertiary hydroxyl in the indole portion of the dimeric alkaloid is not acetylated[3].



I, $R_1 = OCH_3$; $R_2 = H$; $R_3 = OCOCH_3$; $R_4 = COOCH_3$;
II, $R_1 = OCH_3$; $R_2 = H$; $R_3 = H$; $R_4 = COOCH_3$;
III, $R_1 = OCH_3$; $R_2 = COCH_3$; $R_3 = OCOCH_3$; $R_4 = COOCH_3$
IV, $R_1 = OCH_3$; $R_2 = COCH_3$; $R_3 = H$; $R_4 = COOCH_3$
V, $R_1 = NHNH_2$; $R_2 = H$; $R_3 = H$; $R_4 = H$;
VI, $R_1 = NHNH_2$; $R_2 = H$; $R_3 = OH$; $R_4 = H$.

Table II. High resolution mass spectral data of desacetoxy-VLB-hydrazide V.



(VII)

(VIII)

Calcd. for: $C_{41}H_{54}N_6O_4$ (V)	694.4209
Found: M^+	694.4206
Calcd. for: $C_{32}H_{35}N_3O_2$ (VII)	493.2729
Found:	493.2757
Calcd. for: $C_9H_{16}NO$ (VIII)	154.1232
Found:	154.1210

The mass spectrum of II indicated $M^+ = 752$, corresponding to an empirical composition of $C_{44}H_{56}N_4O_7$ also in agreement with microanalyses (see experimental). Since this type of alkaloid undergoes in the mass spectrometer a facile methylation (by the carbomethoxyl group at C-18′) resulting in an increase of molecular ion of 14 mass units, decarbomethoxy hydrazide (V) was prepared in analogy to the corresponding derivative of VLB[4,5] (VI). The high resolution mass spectral data obtained on this derivative confirmed the conclusion that (II) differed from VLB in the absence of the acetoxy group at C-4 of the indoline moiety of the alkaloid (Table II). Of particular significance was the absence of *m/e* 509[5] corresponding to the ion containing hydroxyl at C-4 in VLB hydrazide (VI), and in its place, the presence of *m/e* 493 (VII) representing the corresponding fragment in desacetoxy VLB hydrazide (V). The ion *m/e* 154 was derived from the quinuclidine portion of the indole moiety of dimeric alkaloids[5] of this type with a tertiary hydroxyl at C-4′ (VIII).

Experimental. Proton magnetic resonance spectra. The spectra in $CDCl_3$ were measured using Me_4Si as an internal standard and were recorded on Varian HA-100 MHz instrument. High resolution mass spectra were recorded using a CEC high resolution model 21-110 instrument.

A solution of 20.0 g of impure VLB in benzene was chromatographed over 600 g of alumina (Activity III). After eluting with 4.5 l of ⌀H, fractions of 100 ml were collected and examined by TLC. (Silica Merck, DEA-$CHCl_3$-⌀H; 75:50:100, Dragendorff Reagent; in this system Rf VLB = 0.34 and Rf (II) = 0.37). Fractions No. 5–24 gave 3 g of crude alkaloid. After crystallization from CH_3OH this material still contained traces of VLB. The final purification was achieved by chromatography on Silica (7729) using the solvent mixture used for TLC (vide supra). In a typical run 500 mg of purified alkaloid was chromatographed on Silica (25 g) and fractions of 3ml were collected after discarding the first 36 ml of the eluate. Fractions No. 5, 6, 7 and 8 afforded 320 mg of the alkaloid which readily crystallized from CH_3OH, m.p. 183–190° (d), $[\alpha]_D^{26} = +95.3°$ ($CHCl_3$). For analysis the material was dried on a block at 150° for 1 min (weight loss 6.5%). Calcd for: $C_{44}H_{56}N_4O_7$ (II) C, 70.19; H, 7.50; N, 7.44; O, 14.87. Found: C, 69.71; H, 7.47; N, 7.08; O, 15.00.

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Desacetoxy VLB hydrazide (V) was prepared in boiling absolute ethanol and hydrazine, analogously to that of VLB[3], and crystallized from CH_2Cl_2-MeOH, m.p. 202–207° (d). High resolution mass spectrum, $C_{41}H_{54}N_6O_4$ Calcd. 694.4206; Found: 694.4209. Anal. Calcd for: $C_{41}H_{54}N_6O_4 \cdot CH_3OH$. C, 69.39; H, 8.04; N, 11.56. Found: C, 69.73; H, 7.80; N, 11.51.

[6] Acknowledgments. We wish to thank Mr. J. L. OCCOLOWITZ for the high resolution mass spectral data, Mr. T. K. ELZEY for the ^{1}H-NMR spectra, Mr. G. M. MACIAK for microanalyses and Mr. R. J. ARMSTRONG for the isolation of the partially purified alkaloid.

Zusammenfassung. Auf Grund spektroskopischer Untersuchungen konnte die Struktur eines neuen Alkaloids aus *Vinca rosea* L. als Desacetoxy-VLB aufgeklärt werden.

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The Structure of a Polyacetylenic Diol Isolated from *Vernonia appendiculata* Less. (Compositae)

In continuation of our studies on the sesquiterpene lactones of the genus *Vernonia* (Compositae)[1], we were interested in the investigation of the species *Vernonia appendiculata* Less. originating from Madagascar.

Working up of the chloroform extract of the leaves in the usual manner[2] did not yield any sesquiterpene, but led to the isolation of a crystalline substance which is unstable on exposure to light. This new compound, deca-4,6,8-triyne-1,2-diol, $C_{10}H_{10}O_2$, $M^+ \cdot 162$, m.p. 113–116°, $[\alpha]_D$ -13° (c = 1, MeOH:$CHCl_3$ 2:8) has been assigned the structure *1* on the basis of the following evidence: I.R.

$$\underset{10}{CH_3}\text{–}\underset{9}{C}\equiv\underset{8}{C}\text{–}\underset{7}{C}\equiv\underset{6}{C}\text{–}\underset{5}{C}\equiv\underset{4}{C}\text{–}\underset{3}{CH_2}\underset{2}{CH(OH)}\underset{1}{CH_2OH}$$

1

(Nujol): 3300 cm^{-1} (OH) and 2240 cm^{-1} (C≡C)[3]. U.V. (EtOH): λ_{max} 214 (ε 16,000), 238 (ε 980), 252 (ε 630), 268 (ε 570), 285 (ε 570) and 305 nm (ε 630), characteristic for $-(C\equiv C)_3$-system[4]. M.S.: *m/e* 162 ($M^+ \cdot$), and 144 ($M^+ \cdot - H_2O$). ^{1}H N.M.R. ($CDCl_3$–Pyr., D_2O): 1.92 (3H, s.); $-CH_2-CH(OH)-$ 2.56 (2H, d., 6). The chemical shifts due to the vicinal glycol system $-CH(OH)-CH_2-OH$ are observed as two complex signals centered at 3.65 (2H) and 3.95 (1H) which are displaced in the spectrum of the amorphous diacetate, $C_{14}H_{14}O_4$, $[\alpha]_D$-67° (c = 1, $CHCl_3$) to 4.18 (1H, d.d., 12 and 6.0), 4.37 (1H), d.d., 12 and 4) and 5.14 (1H, q.d., 6.0 and 4) (ABX system)[5].

The formation of the diacetate was confirmed by the examination of the mass spectrum: *m/e* 246 ($M^+ \cdot$), 204 ($M^+ \cdot - 42$), 186 ($M^+ \cdot - 60$) and 126 ($M^+ \cdot - 60 \times 2$).

^{13}C N.M.R. (Pyridine-d_5): 10 carbons from pulsed[6] and off-resonance[7] decoupling measurements, ppm from TMS reference: 3.9 (q), 25.4 (t), 60.7 (s)[8], 61.2 (s)[8], 65.4 (s), 66.0 (t), 67.1 (s), 71.2 (d), 76.3 (s) 78.7 (s); assignments based on alcohol substituent effects[9] and alkyne data[9,10]: C_{10}, C_3, C_7, C_6, C_5, C_1, C_8, C_2, C_9, C_4, respectively.

Résumé. La structure d'un diol polyacétylénique isolé de *Vernonia appendiculata* Less. a été déterminée essentiellement par son étude en résonance magnétique nucléaire (H et ^{13}C).

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A Single-Chain Triple Helical Structure in Synthetic Polypeptides

The existence of a single-chain triple helical structure, in which a single polypeptide chain folds back on itself to form a stable collagen-like triple helix, was first suggested for the subunit of *Ascaris* collagen by MCBRIDE and HARRINGTON[1], and for the synthetic polypeptide (Pro-Pro-Gly)$_n$ by ENGEL[2]. On the basis of model building studies, RAMACHANDRAN, DOYLE and BLOUT[3] were able to give the details of such a structure for (Pro-Pro-Gly)$_n$. They suggested that the single-chain triple helix is a possible conformation for polypeptides of the type (X-Y-Gly)$_n$, where X and Y are any amino or imino acids.

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