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): $\lambda_{max}$ 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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[11] Acknowledgments: We are indebted to Professor E. LEDERER for his general interest in this work and to Doctor B. C. DAS for valuable suggestions.

## 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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A backfolded antiparallel structure has also been reported for lamellae of (Pro-Pro-Gly)$_n$ prepared by evaporation from solutions in dioxane-water mixtures[4]. This contrasts with evidence for triple-chain association in (Pro-Pro-Gly)$_n$ of defined molecular weight[5–7], and with the normal fibre orientation observed in other preparations[8]; but the possibility of structural variability cannot be excluded here.

Table I. X-ray data for (Pro-Leu-Gly)$_n$. Three polypeptide chain in triple helix; monoclinic (pseudohexagonal) unit cell $a \simeq b = 12.6$, $c = 28.5$ Å, $\gamma = 115°$

| h | k | l | $d_c$ | $d_o$ | $I_o$ |
|---|---|---|---|---|---|
| 1 | 0 | 0 | 11.40 | 11.5 | vvvs |
| 1 | 0 | 2 | 8.90 | 8.65 | m |
| 1 | 1 | 0 | 6.76 | 6.70 | m |
| 2 | 0 | 0 | 5.70 | 5.65 | vs |
| 2 | 0 | 4 | 4.45 | 4.5 to | |
| 1 | 0 | 6 | 4.38 | | broad vs |
| 2 | 1 | 3 | 4.00 | 4.0 | |
| 1 | 0 | 7 | 3.83 | 3.65 | w |
| 2 | 2 | 0 | 3.38 | 3.32 | w |
| 0 | 0 | 10 | 2.85 | 2.86 | w |
| 2 | 1 | 10 | 2.39 | 2.40 | w |

Table II. X-ray data for (Pro-Leu-Gly)$_n$. Single polypeptide chain in triple helix; monoclinic (pseudohexagonal) unit cell $a \simeq b = 12.6$, $c = 8.6$ Å, $\gamma = 115°$

| h | k | l | $d_c$ | $d_o$ | $I_o$ |
|---|---|---|---|---|---|
| 1 | 0 | 0 | 11.40 | 11.5 | vvvs |
| 0 | 0 | 1 | 8.60 | 8.65 | m |
| 1 | 1 | 0 | 6.76 | 6.70 | m |
| 2 | 0 | 0 | 5.70 | 5.65 | vs |
| 0 | 0 | 2 | 4.30 | 4.5 to | |
| 1 | 0 | 2 | 4.02 | 4.0 | broad vs |
| 1 | 1 | 2 | 3.63 | 3.65 | w |
| 2 | 2 | 0 | 3.38 | 3.32 | w |
| 0 | 0 | 3 | 2.87 | 2.86 | w |
| 2 | 1 | 3 | 2.40 | 2.40 | w |

Table III. X-ray data for (Pro-Phe-Gly)$_n$. Single polypeptide chain in triple helix; hexagonal unit cell $a = 15.0$, $c = 8.7$ Å

| h | k | l | $d_c$ | $d_o$ | $I_o$ |
|---|---|---|---|---|---|
| 1 | 0 | 0 | 13.00 | 13.00 | vvvs |
| 0 | 0 | 1 | 8.70 | 8.75 | m |
| 1 | 0 | 1 | 7.23 | 7.18 | w |
| 1 | 2 | 0 | 4.91 | 4.96 | s |
| 0 | 0 | 2 | 4.35 | 4.38 | vs |
| 1 | 1 | 2 | 3.76 | 3.80 | m |
| 2 | 0 | 2 | 3.62 | 3.60 | m |
| 0 | 0 | 3 | 2.90 | 2.87 | w |

Recently, we synthesized the sequential polypeptides (Pro-Leu-Gly)$_n$ and (Pro-Phe-Gly)$_n$, and were able to demonstrate by circular dichroism measurements that in certain conditions these polymers assume a triple helix structure in solution. As shown in Table I for unoriented films and powders of (Pro-Leu-Gly)$_n$, the spacings and intensity distribution are remarkably similar to those of unstretched collagen[9].

We have been able to measure about 9 spacings and to estimate their intensities, as reported in Table I. All the reflections could be approximately indexed on the basis of monoclinic (pseudo-hexagonal) packing in a unit cell with $a \simeq b = 12.6$ and $c = 28.5$ Å, $\gamma = 115°$. On this basis, as in collagen, there is a relatively strong 2.85 Å reflection on the 10th layer line, indicating a helix with an axial translation of 2.85 Å per unit of structure and approximately 10 units in an integral number of turns. We have been forced, however, to index the 8.6 Å reflection as (102), which contradicts helical diffraction theory[10], as this requires for a collagen-like structure relatively strong near meridional reflections only on the 3rd, 7th, 9th and 10th layer lines. Therefore, we have examined the X-ray diffraction pattern for any indication of an arrangement in which a single molecule, by coiling back on itself, might form a triple-stranded structure of 2 parallel and 1 antiparallel strands.

This model (Table II) is supported by the presence of the 8.6 Å reflection. In addition, a 4.3 Å reflection could be present but hidden by the broad band in this region (4.0—4.5 Å).

In the case of (Pro-Phe-Gly)$_n$ (Table III), the same model is more strongly supported by the presence of the 8.75, 4.38, 2.87 Å reflections, which can be indexed as (001), (002) and (003), respectively; the molecules being packed in an hexagonal lattice with $a = 15.0$, $c = 8.7$ Å.

Interestingly, we have not obtained similar results for the isomeric sequences (Leu-Pro-Gly)$_n$ and (Phe-Pro-Gly)$_n$. It seems, therefore, that the presence of proline in position 1 is required, but not sufficient for the stabilization of the folded conformation, since (Pro-Ala-Gly)$_n$[11] does not adopt this kind of a structure. Furthermore, the nature of the residue in position 2 seems to be important. In fact, the side chain should be both apolar and bulky, because (Pro-Ser-Gly)$_n$[12], (Pro-Ala-Gly)$_n$[11] and (Pro-Gly-Gly)$_n$[13] do not have a single chain of triple helical structure. A possible explanation for this finding could be the stabilization of the whole structure by the increased van der Waals energy resulting from a closer packing of the residues in position 2 lying on different chain segments.


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*Riassunto.* Misure di diffrazione ai Raggi-X sui polipeptidi sintetici (Pro-Leu-Gly)$_n$ e (Pro-Phe-Gly)$_n$ permettono di suggerire una struttura originata dall'avvolgimento di una singola catena a formare una tripla elica. Il confronto tra i dati ottenuti dagli autori e quelli riportati in letteratura indica che la presenza di prolina in posizione 1 è necessaria ma non sufficiente e che inoltre è importante la natura del residuo in posizione 2.

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## Sex Pheromones of the Armyworm Moth, *Spodoptera exempta* (Wlk.

The armyworm, *Spodoptera exempta* (Wlk.) (Lepidoptera, Noctuidae) is a serious pest of cereals and grasses which occurs in Africa, India, Malaysia, Indonesia and Australia. A synthetic sex pheromone would provide a valuable aid to its control. We have found that virgin female *S. exempta* produce two compounds which are potent olfactory stimulants for the male moth. They have been identified as (Z)-9-tetradecen-1-yl acetate (I) and (Z)-9,(E)-12-tetradecadien-1-yl acetate (II) and have been shown to attract male moths under laboratory conditions.

As only a small number of moths was available, gas chromatography (GC) combined with electroantennogram (EAG) recording[1] was used for the identification of the pheromones. Extracts of 1-day-old virgin female and male moths were prepared after a 3 h darkness treatment. The last 3 to 4 abdominal segments were clipped and extracted with dichloromethane for 15 min at room temperature without maceration; the solvent was removed by pipette and concentrated to 1 tip equivalent/µl.

Both male and female moth preparations were made for EAG recording. The extracts were chromatographed on polar and non-polar packed GC columns and the column effluent split 75% to the flame ionisation detector and 25% to the test insect's antenna; GC peaks and EAG responses were recorded simultaneously. The female moth did not respond to any components of either male or female extract (c.f.[2]). Male moths responded only to female extract: two responses were obtained to the effluent from a polar column, but from a non-polar column the 2 olfactory stimulants (designated S(i) and S(ii)) emerged as a single peak (Table I). The amounts of the 2 compounds in a female tip equivalent were 4 ng S(i) and approximately 0.2 ng S(ii).

As preliminary examination of their GC behaviour suggested that S(i) and S(ii) were acetates of $C_{14}$ unsaturated alcohols, they were compared directly with synthetic samples of the known sex pheromones of other *Spodoptera* species which are all compounds of this type[3–6] (Table II). The results indicated that S(i) was a tetradecenyl acetate and S(ii) was a tetradecadienyl acetate in which the two double bonds were methylene interrupted. In an attempt to establish the positions and configurations of the double bonds in the two compounds, female tip extract (0.5 µl), a range of tetradecenyl acetate isomers ((Z)- and (E)-7, -8, -9, -10, -11 and -12) and all 4 stereoisomers of 9,12-tetradecadienyl acetate were run through the GC-EAG link on support-coated open tubular columns (50 ft Carbowax 20M, Apiezon L and DEGS run isothermally at 150, 165 and 141 °C respectively, with a helium flow of 4 ml/min). All synthetic samples were chromatographed at a loading of 2 ng. Only (E)-10-tetradecenyl acetate and (Z)-9-tetradecenyl acetate (I) co-chromatographed with S(i), and only the (Z)-9 isomer elicited an EAG response at this loading. Only

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Table I. GC retention temperatures of the olfactory stimulants S(i) and S(ii) in female moth extract and corresponding male antennal responses

| Sample | Retention temperature (°C) Column A | EAG response (mV) | Retention temperature (°C) Column B | EAG response (mV) |
|---|---|---|---|---|
| Dodecanyl acetate | 155.0 | | 147.6 | |
| Tetradecanyl acetate | 172.5 | | 164.6 | |
| Hexadecanyl acetate | 191.4 | | 182.8 | |
| S(i) 4 ng | 175.8 | 1.3 | 163.0 | 1.7 |
| S(ii) ca. 0.2 ng | 183.0 | 0.4 | | |

Column A: 2½% Carbowax 20 *M* on Chromosorb G AW DCMS, temperature programmed at 4 °C/min from 120–200 °C with $N_2$ flow 25 ml/min. Column B: SE 30 (other conditions as for Column A).