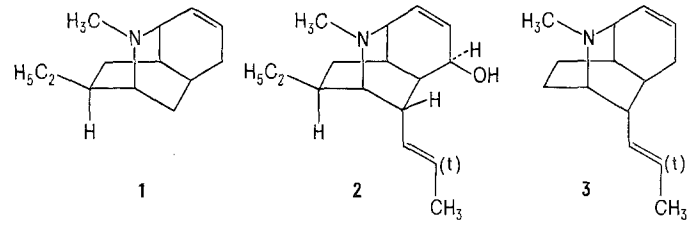
Scheme 1



MS-30) revealed by exact mass measurement cannivonine **1** → 191.1668 a.m.u., **2** → 247.1979 a.m.u., and **3** → 203.1699 a.m.u., which correspond to the respective formulas $C_{13}H_{21}N$ (191.1669), $C_{16}H_{25}NO$ (247.1930), and $C_{14}H_{21}N$ (203.1669).

Medium resolution mass spectrometry afforded a base peak at $C_{13}H_{17}N$, or M-59 a.m.u. for cannivonine **2**. The IR-spectrum showed OH absorption at 3217 and unsaturation absorption at 1632 and 1662 cm^{-1} and the UV-spectrum shows 2 olefinic absorptions (208 nm and 238 nm). The NMR-spectrum (VARIAN T-60) consisted of only a few signals, the majority of protons being located in the 1.7–3.3 ppm region (Table).

From these data, the tricyclic structur of 2-methyl-10-ethyl-11-prop-2-enyl azatricyclo [5.3.1.0]^{3,8} undec-4-en-6-ol, isproposed for cannivonine **2** (Scheme). The two other Cannivonines, **1** and **3**, are likely to possess the structures presented in Scheme 1.

The assignment of hydroxyl, propenyl, and ethyl group, as well as double bond position on the skeleton was made using the NMR shift reagent technique. We are conscious of the fact that the coupling constants calculated for our product are not the true product coupling constants but the coupling constants of the resulting complex[4–6]. The best expansion of the spectrum was obtained using a Varian HR-220MHz NMR-spectrometer, together with the shift reagent E-FOD. The values of all coupling constants permitted the establishment of the stereochemistry of C(6), C(10) and C(11). Unfortunately, the molecule of cannivonine **2** is not as rigid as it would seem to be. The cylcohexene side of the molecule can adopt 2 conformations, half-chair (HC) or boat (B) which changes considerably the conformation of the whole molecule. Theoretical calculation of angles (using a Karplus type equation) permitted the elimination of the boat structure. The Table contains the average angle values for the 2 possible conformations of the cyclohexene ring. The differences between calculated values of vicinal coupling constants and observed values can be attributed to the presence of the shift reagent. However, they are in good agreement with the half-chair conformation values of a cylcohexene ring.

The nitrogen lone pair is pushed to the inside of the molecule, which is its more natural position (endo).

Some long-range coupling constants were also studied. The absence of a 'W' coupling between the N-CH_3 protons and the 1-C proton shows that the nitrogen doublet is endo orientated. The other long-range coupling constant – homoallylic, 1.7 Hz – between H-3 and H-6 proved that these protons are *cis*. Some known pseudo-axial, pseudoequatorial homoallylic coupling constants are of the same order[7–10].

Resumé. La séparation de trois nouveaux alcaloïdes de canneberges – cannivonines **1**, **2** et **3** – a été effectuée à l'aide de solvants appropriés et de la chromatographie sur couche mince préparative. Une identification des structures des trois alcaloïdes est proposée.

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New Utilizations of the Phenacyloxy Protecting Group in Peptide Synthesis

The reaction of 2-bromoacetophenone and its derivatives with phenolates[1] and carboxylates[2,3] to form the corresponding phenacyloxy (PAO) ethers and esters, is well known. The preparation of such derivatives is a simple procedure usually providing a crystalline product in good yield. As a consequence, this reaction became very useful for the systematic identification of carboxylic acids[2,3].

More recently, this group was introduced into peptide synthesis by Stelakatos et al.[4] as carboxy protecting group. PAO ethers and esters are quite stable to acidic hydrolysis[5] and acidolysis[4]. Their reductive cleavage, however, can be carried out under mild conditions e.g. with zinc dust and acetic acid[5]. This approach has been suggested by Trudelle[6] for activation of the *o*-phenacyloxy-phenyl carboxy protecting group to obtain the active *o*-hydroxyphenyl esters.

In view of the cyanhydrine formation of acetophenone investigated a long time ago[7], it came to our attention

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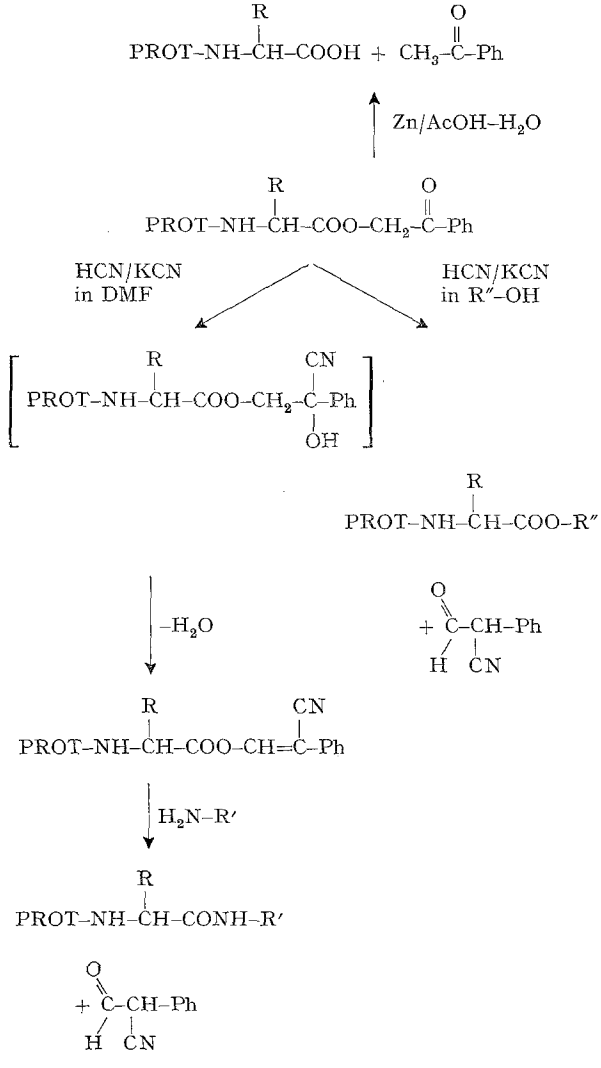
that the PAO protected carboxy groups could be converted into useful active esters of the vinyl ester type. Preliminary investigations in our laboratory have shown this to be indeed the case. The PAO groups react with hydrogen cyanide in the presence of a base (e.g. KCN) to form the 2-cyano-2-phenyl-vinyl (CPV) derivative. In 0.05 mmole/ml concentration of t-BOC-L-Ala-OCPV and an amine (H-Gly-OMe, Bzl-NH_2) the corresponding amide was formed. The reaction was carried out at room temperature in 12 h reaction time. The products were obtained in about 50% yield (monitored by quantitative TLC, in situ at 208 nm on a Zeiss Chromatogram Spectrophotometer).

For the formation of the CPV ester, DMF turned out to be an adequate solvent. Using alcohols as solvent, complete transesterification occurs. Moreover, in the presence of water, unprotected carboxylic acid is recovered.

During the investigation of the reactions described above, the chromatographic and photometric properties of the products were always compared to those of authentic compounds purchased or prepared by conventional methods (Table I). The Scheme shows some of the reactions proceding through the CPV ester intermediate.



R, R′ and R″, H or alkyl group.
PROT, amino protecting group (e.g. Z or t-BOC).

Table I. Chromatographic properties of some amino acid derivatives

Derivatives	Rf[a] values in solvent system A	B	Method of preparation (ref.)
Z–Gly–OPAO	0.36	0.56	Z[8], PAO[5]
t–BOC–L–Ala–OPAO	0.44	0.62	t–BOC[9], PAO[5]
t–BOC–L–Glu(OBzl)–OPAO	0.46	0.66	Bzl[10], t–BOC[11], PAO[5]
t–BOC–L–Lys(Z)–OPAO	0.41	0.60	Z[12], t–BOC[11], PAO[5]
Z–Gly–OCPV[b]	0.36 0.40	0.59 0.65	CPV[7]
t–BOC–L–Ala–OCPV[b]	0.37 0.44	0.59 0.66	CPV[7]
t–BOC–L–Glu(OBzl)–OCPV[b]	0.47 0.52	0.69 0.73	CPV[7]
t–BOC–L–Lys(Z)–OCPV[b]	0.40 0.44	0.65 0.70	CPV[7]
t–BOC–Ala–OMe	0.50	0.63	Me[13]
t–BOC–L–Ala–OEt	0.52	0.66	Et[13]
t–BOC–L–Ala–OBzl	0.54	0.70	Bzl[14]
t–BOC–L–Ala–NH–Bzl	0.26	0.50	[15]
t–BOC–L–Ala–Gly–OMe	0.29	0.45	[15]

[a] On precoated silica gel plates (E. Merck, Darmstadt/West Germany) in the solvent A) Benzene-pyridine (9:1 v/v). B) Chloroform-acetone-acetic acid (9:1:0.5 v/v). [b] Due to syn-anti isomery 2 forms appear.

Table II. Physical constants of some of the new amino acid derivatives

Derivative	m.p.[a]	$[\alpha]_D^{25}$[b]
Z–Gly–OPAO	99 °C	
t–BOC–L–Ala–OPAO	122 °C	−4.90°
t–BOC–L–Glu(OBzl)–OPAO	109 °C	−1.72°
t–BOC–L–Lys(Z)–OPAO	92°	−1.71°

[a] After recrystallization from 96% ethyl alcohol. [b] c = 1 in ethyl acetate.

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It is remarkable that the reaction conditions specifically provide an activated ester without affecting the most common protecting groups.

In conclusion we find that the use of phenacyloxy protecting group in peptide synthesis has the advantage that crystalline non-racemized product may be obtained. Considering the mild reaction conditions of the conversion into the activated ester, no racemization may be expected even by using the phenacyloxy intermediates for coupling of fragment peptides.

The present preliminary communication will be followed by a paper with the experimental details and with further investigations on amino acid and peptide derivatives containing the PAO and CPV group.

Zusammenfassung. Es wird über weitere Verwendungsmöglichkeiten der Phenacyloxy-Gruppe in der Peptidsynthese berichtet. Phenacyloxyester reagieren leicht mit Hydrogencyanid unter Bildung der entsprechenden 2-Cyano-2-phenyl-vinyl-ester, welch letztere Derivate sich als gute Acylierungsmittel erwiesen.

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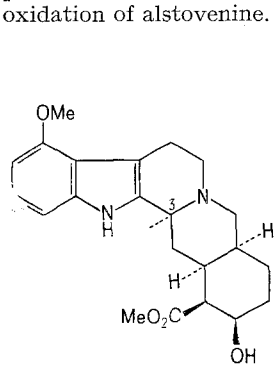
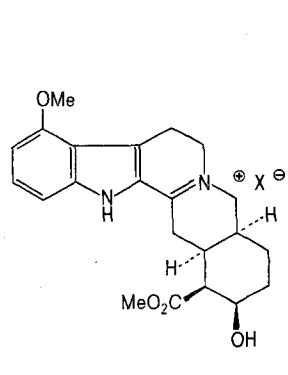
Quaternary Alkaloid of the Bark of *Alstonia venenata* R. Br.

To date, 8 tertiary indole alkaloids[1-4] and an amine oxide, venoxidine[5], have been reported from the bark of *Alstonia venenata* R. Br., a plant which is used in the treatment of insanity and epilepsy in the Indian system of medicine[6]. Search for water-soluble bases in the bark of this plant has resulted in the isolation of a yellow quaternary alkaloid as its chloride, $C_{22}H_{27}N_2O_4Cl$, m.p. 216° (dec.), λ ethanol 207, 252 infl., 256, 348 and 400 nm (log ε, 4.33, 4.19, 4.21, 4.26 and 3.83). The isolation of the alkaloid was achieved by precipitation of the total water-soluble base as Mayer's complex, regeneration of the base chloride by treatment with IRA 400 (Cl^- form) and chromatography over silica gel. The alkaloid as its chloride salt, is slightly hygroscopic in nature but gives fairly stable perchlorate, m.p. 243–244° (dec.) and picrate, m.p. 257–258° (dec.). The UV-spectrum of the alkaloid chloride shows a reversible acid-base shift, λ ethanol/OH^- 230, 297, 308 and 322 nm (log ε, 4.40, 4.29, 4.33 and 4.19), very much like that of 3-dehydroyohimbine, as has been observed by GODTFREDSEN and VANGEDAL[7]. The IR spectral bands at 1635, 1580 and 1552 cm^{-1} of the alkaloid perchlorate are also suggestive of a dihydro-β-carbolinium moiety[8] in the molecule. In conformity with these observations, the alkaloid on reduction with sodium borohydride furnished a tertiary base, $C_{22}H_{28}N_2O_4$, m.p. 170–172°, identical in all respects with alstovenine[1] (I) or isovenenatine[2]. Accordingly, the quaternary base was considered to be Δ^3-alstovenine (II), additional proof of which was secured by direct comparison of the alkaloid perchlorate with the one obtained by mercuric acetate oxidation of alstovenine.



(I)



(II)

The 60 MHz PMR-spectrum of the alkaloid chloride, taken in D_2O, shows a 6 proton methoxy singlet at δ 4.00 (Ar-OMe and COOMe) and 3 aromatic protons spread over a region of δ 6.65–7.65. Unlike alstovenine, which exhibits three vicinal aromatic protons as complex multiplets, these protons appear as three apparent doublets at δ 6.75, 7.15 and 7.53 (J = 8.5 Hz). The simplification of the splitting pattern is presumably due to the electron withdrawing $-C=N^+$ system in conjugation with the indole nucleus which increases the nonequivalency of the protons concerned.

Preparations of the quaternary alkaloid (II), both by oxidation of alstovenine with mercuric acetate[1,2] and venenatine (C-3 epimer of I) with tert. butyl hypochlorite[2], are on record but we have reckoned that such preparation can also be made quite satisfactorily by oxidation of venenatine with hydrogen peroxide in acetic acid. While oxidation of venenatine with hydrogen peroxide in acetic acid under controlled condition is reported to yield venoxidine[5], 3-dehydroalstovenine is the major isolable product if the reaction is carried out at room temperature for 72 h or at waterbath temperature for a short time.

Δ^3-Alstovenine is the second major alkaloid (yield, 0.2%) of the bark of *Alstonia venenata* and is considered responsible for the yellow tint of the latter[9].

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