$N_{(1)}$-METHYL-2β,16β-DIHYDROAKUAMMICINE-$N_{(4)}$ METHOCHLORIDE AND EPIPLEIOCARPAMINE $N_{(4)}$-OXIDE, TWO NEW ALKALOIDS FROM *Vinca minor* L.*

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Two new quaternary alkaloids, $N_{(1)}$-methyl-2β,16β-dihydroakuammicine $N_{(4)}$-methochloride and epipleiocarpamine $N_{(4)}$-oxide, were isolated from the leaves of *Vinca minor* L. Their constitution and configuration were established on the basis of spectral evidence and correlation.

Although a considerable attention has been paid to alkaloids of *Vinca minor* and some of them are used in human medicine, quaternary alkaloids of this plant remained neglected as yet.

The portion of pH 7, obtained by extraction of mother liquors after removal of vincamine[1] with McIlvain buffer solution, was worked up to give two new alkaloids. Alkaloid *I* showed in its UV spectrum absorption maxima at 241, 293 and 312 nm (log ε 3·74, 3·49, 2·34) characteristic of the strychnine type of alkaloids[2]. High resolution mass spectrum displayed the peak of molecular ion at *m/e* 338·1998 (for $C_{21}H_{26}N_2O_2$ calculated 338·1994) and further peaks of ions at 307 (M − OCH_3, $C_{20}H_{23}N_2O$), 265 (M − CH_2COOCH_3), 194 (base peak, $C_{11}H_{16}NO_2$), 158 ($C_{11}H_{12}N$), 157 ($C_{11}H_{11}N$), 144 ($C_{10}H_{10}N$) and 139 ($C_8H_{11}O_2$) diagnostic of 2,16-dihydroakuammicine[3]. The peaks of ions of substance *I* associated with the indole moiety of the molecule were shifted by 14 mass units towards higher values, those having the indole moiety split off, appeared at the same values as the corresponding peaks of 2,16-dihydroakuammicine. The IR spectrum revealed vibration bands of four neighbouring protons attached to a benzene ring (760 cm^{-1}), of an ethylidene grouping (837, 1674 cm^{-1}), an ester group at $C_{(16)}$ (1158, 1735, 1728 cm^{-1}) (ref.[2,4]) and of an indole backbone (1456, 1626 cm^{-1}). The ^{1}H-NMR spectrum (ppm, δ scale) contains signals of protons of an aromatic ring (7·10−6·76, m) of an indoline grouping (6·05, d, J = 8 Hz), an ethylidene (5·75, q), a methoxycarbonyl (3·77, s),

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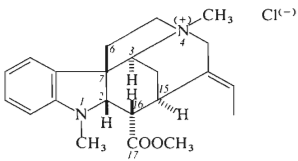
an $N_{(1)}$—CH_3 and of $N_{(4)}$—CH_3 (3·31, s and 2·92, s) groups, of a methylene in the neighbourhood of which no protons are present (2·75, s) and a methyl group of an ethylidene grouping (1·57, d, J = 7 Hz); the signal of the proton adjacent to methoxycarbonyl group resonates in the region overlapped by the intense signal of the solvent. The appearance of a signal due to protons of the $N_{(4)}$-methyl group in the ^{1}H-NMR spectrum and also the presence of peaks of chloromethane at m/e 50 and 52 in the mass spectrum prove the alkaloid to be in form of a quaternary salt. Basing upon arguments presented one can postulate, excepting the configuration, the structural formula *I* for this alkaloid.

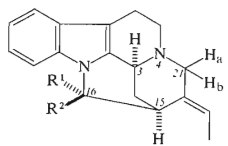
The configuration at five chiral centres was adduced as follows: The fusion of rings B, C and E in the series of indoline alkaloids was studied on the strychnine and dihydroaspidospermatidine types of alkaloids[5]; it has been found that both types have ORD curves almost enatiomeric. Their stereostructure was confirmed by chemical correlation. The strychnine type is characterized by a positive, dihydroaspidospermatidine one by a negative Cotton effects. Since the ORD curve of alkaloid *I* traces the course indicative of the strychnine type of alkaloids in the whole spectral range, the proton at the chiral centre $C_{(2)}$ should be β-oriented and consequently, the bond towards $C_{(6)}$ in ring E, coming from the chiral centre $C_{(7)}$ also β-oriented. This spatial arrangement allow exclusively the α-orientation of protons at the further two chiral centres $C_{(3)}$ and $C_{(15)}$. Configuration of the $C_{(16)}$-methoxycarbonyl group can be determined by means of IR spectrum. It has been found[2], on the basis of chemical correlations of synthetically prepared 2,16-dihydroakuammicines with fluorocurarine, that the $C_{(16)}$-methoxycarbonyl group in an equatorial α-position interacts with the $N_{(1)}$-proton, this being seen in splitting of the $\tilde{\nu}$(C=O) absorption band; the axial orientation of the $C_{(16)}$ epimer does not influence the shape of this band. Splitting of the IR absorption band of alkaloid *I* indicates the α-orientation of the $C_{(16)}$-methoxycarbonyl group. All these findings favour the structure methyl (1,4-dimethyl-2β,16β-cur-16-en-17-oate)-4-chloride for alkaloid *I*.

Alkaloid *II* showed in the UV light absorption maxima at 232 and 285 nm (log ε 4·2, 3·9) typical of the C-mavacurine type of alkaloids[5]. The mass spectrum revealed the peak of molecular ion at m/e 338·1625 (for $C_{20}H_{22}N_2O_3$ calculated 338·1630) and other peaks at m/e 322 (M − 16, $C_{20}H_{22}N_2O_2$), 321 (M − 17), 263 (M − O—COOCH$_3$, $C_{18}H_{19}N_2$), and 180 indicative of the afore-mentioned type of indole alkaloids[6]. The appearance of M − 16 and M − 17 peaks is ascribed to the presence of N-oxides[8]. The spectrum of alkaloid *II* labelled in the ion source with [O—^{2}H] ethanol did not undergo an alteration; this confirmed that no active hydrogen was present in the molecule. The IR spectrum displayed vibration bands of four neighbouring protons of an aromatic skeleton (752 cm^{-1}), of an ethylidene grouping (820, 1 670 cm^{-1}), an ester group (1 162, 1 730, 1 737 cm^{-1}) and of an indole backbone (1 443, 1 625 cm^{-1}). The ^{1}H-NMR spectrum confirmed the presence of groups

already identified on the basis of preceding data (7·57 – 7·10, m, protons of an aromatic ring; 5·16, q, CH_3—C<u>H</u>=; 1·62, d, J = 7 Hz, C<u>H</u>$_3$—CH=; 3·72, s, $COOCH_3$) and displayed further protons useful for the structure elucidation: the $C_{(16)}$ proton partly overlapped by the signal of the solvent appeared at 4·72, the $C_{(15)}$ at 3·86, the $C_{(3)}$ at 3·65, the $C_{(21b)}$ at 2·49 and the $C_{(21a)}$ protons at 0·93. These arguments entitle to ascribe the structural formula *II* (excepting the stereochemistry) to the alkaloid under study. Configurations at $C_{(3)}$ and $C_{(15)}$ are given by the only one possible *cis* fusion of rings C, D, E, the configuration at $C_{(16)}$ was deduced on the basis of ^{1}H-NMR spectral data. The model substance for determination of the spatial arrangement at this chiral centre was the pair of alkaloids epipleiocarpamine (*III*) and pleiocarpamine (*IV*), the ^{1}H-NMR spectra of which significantly differ[7]; due to an interaction of the β-equatorial proton at $C_{(16)}$ of epipleicarpamine with the opposite β-proton at $C_{(21a)}$ a downfield shift by 0·52 and 0·75 ppm with respect to pleiocarpamine takes place. The position of signals of the individual protons of alkaloid *II* coincided with data reported[7] for epipleiocarpamine.



I



II, $R^1 = COOCH_3$, R^2 = H,
III, $R^1 = COOCH_3$, R^2 = H $N_{(4)}$-oxide
IV, R^1 = H, $R^2 = COOCH_3$

To verify the correctness of the suggested structure, we reduced *II* with sodium borohydride; since the physical constants and spectral data of the product of hydrogenolysis were found to be identical with *III*, alkaloid *II* was assigned the structure epipleiocarpamine $N_{(4)}$-oxide.

EXPERIMENTAL

Melting points were determined on a Kofler micro hot-stage, optical rotation was measured with a Bendix-Ericson, model 143 D, polarimeter in methanol. Following apparatuses were employed for measuring spectra: UV/ORD-5 Jasco for ORD in methanol, Roussel–Jouan dichrograph, model 185/II, for CD in methanol, Beckman DB-GT for UV in methanol, Perkin–Elmer, model 457, for IR in KBr, AEI MS, model 902, for mass spectra, and Tesla BS, model 487 B, operating at 80 MHz for ^{1}H-NMR spectra in ^2H_2O with addition of sodium 3-(trimethylsilyl)-

propionate as an internal reference substance. Alumina (Merck, neutral) for column chromatography, activity grade III, and silica gel G (Woelm for TLC) were the carriers; solvent systems $CH_3OH—NH_4OH—H_2O$ 8 : 1 : 1 (S_1), 7 : 1 : 2 (S_2), 5 : 2 : 3 (S_3). Visualization with Dragendorf reagent.

Isolation of Alkaloids

The mixture of alkaloids (15 g), obtained by extraction of the chloroform solution of mother liquors of *Vinca minor* L. after removal of vincamine[9] with McIlvain pH 7·0 buffer solution (10 . . 100 ml), was precipitated with Mayer's reagent at pH 4, filtered off, washed with water, dissolved in acetone–methanol–water 6 : 2 : 1 and passed the Amberlite IRA-402 column in $Cl^{(-)}$form. The chlorides obtained by this procedure were separated by means of column chromatography using benzene with increasing amount of methanol as eluent. Fractions benzene–2% methanol afforded *II*, benzene–4% methanol *I*.

$N_{(1)}$-Methyl-2β,16β-dihydroakuammicine $N_{(4)}$-Methochloride (*I*)

$C_{22}H_{29}ClN_2O_2$, 1·1 g, amorphous, $[\alpha]_D^{22}$ +6° (*c* 1), R_F 0·24 (S_2); iodide, m.p. 310°C (acetone), R_F 0·37 (S_3); tartrate, m.p. 158—162°C (ethanol), $[\alpha]_D^{22}$ +4° (*c* 1), R_F 0·34 (S_1). ORD: 258 nm, +25100; 233, —34500; CD: 400 nm, Δε 0, 335, +0·04; 322, 0; 290, —0·63; 267, 0; 244, +4·6; 224, 0; 219, —0·61; 215, 0.

Epipleiocarpamine $N_{(4)}$-Oxide (*II*)

$C_{20}H_{22}N_2O_3$, 225 mg, amorphous, R_F 0·81 (S_1); tartrate, m.p. 150—153°C (ethanol), $[\alpha]_D^{23}$ +4·5°(*c* 1), R_F 0·83 (S_1).

Epipleiocarpamine (*III*)

Sodium borohydride (8·6 mg) was added to a solution of epipleiocarpamine $N_{(4)}$-oxide (*II*, 100 mg) in methanol (3 ml). The mixture was stirred at room temperature for 8 h, allowed to stand overnight, the excess of hydride was decomposed with water (0·5 ml) and methanol removed *in vacuo*; 1M-H_2SO_4 was added to the residue to pH 5, and after alkalization with ammonium hydroxide the solution was extracted with chloroform. The product of hydrogenolysis revealed identical ^{1}H-NMR and mass[7] spectra with those of epipleiocarpamine.

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