PROTOBERBERINE ALKALOIDS FROM THE BARK OF *Enantia chlorantha*

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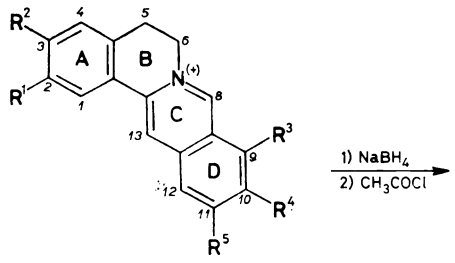
Four protoberberine alkaloids, palmatine (*Ia*), jatrorrhizine (*Ib*), columbamine (*Ic*) and pseudocolumbamine (*Id*), were isolated from the bark of *Enantia chlorantha* and characterized. The corresponding acetylated tetrahydro derivatives (*IIa*—*IId*) were also identified.

Recent investigations with experimentally traumatized test animals have proven that protoberberines from the bark of *Enantia chlorantha* (*Annonaceae*) have preventive and curative effects on artificially provoked liver injury[1-3]. Protoberberine alkaloids have the general structure (*I*). They differ from one another with respect to the number and position of various oxygen functions. Natural tetrahydroprotoberberines have the *S*-configuration, as shown by circular dichroism measurements[4] and the configuration has shown to be independent of the substitution pattern[4].

This paper reports the characterization of protoberberines (*Ia*—*Id*) in an extract from the bark of *Enantia chlorantha* and the finding of pseudocolumbamine (*Id*), not previously found in *E. chlorantha* as one of the main protoberberines in the extract. The analyses of the corresponding acetylated tetrahydro derivatives *IIa*—*IId*



I *II*

Ia, $R^1 = R^2 = R^3 = R^4 = OCH_3$; $R^5 = H$
Ib, $R^1 = R^3 = R^4 = OCH_3$; $R^2 = OH$; $R^5 = H$
Ic, $R^2 = R^3 = R^4 = OCH_3$; $R^1 = OH$; $R^5 = H$
Id, $R^2 = R^4 = R^5 = OCH_3$; $R^1 = OH$; $R^3 = H$

IIa, $R^1 = R^2 = R^3 = R^4 = OCH_3$; $R^5 = H$
IIb, $R^1 = R^3 = R^4 = OCH_3$; $R^2 = CH_3CO_2$; $R^5 = H$
IIc, $R^2 = R^3 = R^4 = OCH_3$; $R^1 = CH_3CO_2$; $R^5 = H$
IId, $R^2 = R^4 = R^5 = OCH_3$; $R^1 = CH_3CO_2$; $R^3 = H$

are included because they serve as proof for the characterization of the natural protoberberines.

The gas chromatogram of the hydrogenated and acetylated bark extract displayed four major peaks originating from tetrahydroprotoberberines as shown by their mass spectra. The second largest peak was shown to be composed of two overlapping peaks (See Experimental). Thus, five different tetrahydroprotoberberines were detected by GC-MS analyses and their relative amounts determined by GC and ^{1}H NMR spectroscopy. Three of these (*IIb*–*IId*) were monoacetylated which confirms the presence of one hydroxyl group in the natural protoberberines. The main components consisted of tetrahydropalmatine (*IIa*, c. 63%), 3-O-acetyltetrahydrojatrorrhizine (*IIb*, c. 19%) and 2-O-acetyltetrahydrocolumbamine (*IIc*, c. 8%). The structures of *IIa* and *IIb* were confirmed by comparison with authentic samples after hydrogenation and acetylation as above (GC-MS and ^{1}H NMR). The structure of *IIc* was deduced from the interpretation of its ^{1}H NMR and mass spectra. Moreover, *IIc* was formed on acetylation of the tetrahydro derivative of *Ic*, as shown by GC-MS analyses and ^{1}H NMR spectroscopy. Their precursors *i.e.* palmatine (*Ia*), jatrorrhizine (*Ib*) and columbamine (*Ic*) were isolated from the extract by TLC and analyzed by ^{1}H NMR spectroscopy. The spectra of *Ia* and *Ib* were identical with those of authentic samples. The interpretation of the ^{1}H NMR spectrum of *Ic* is based on comparison with the spectra of *Ia* and *Ib* and on published ^{1}H NMR data[5].

The fourth abundant component (9% by GC) in the hydrogenated and acetylated extract was isolated by preparative TLC and was found to be identical with *IId*. We thus concluded that *IId* was formed from pseudocolumbamine (*Id*), a protoberberine not previously found in extracts of *E. chlorantha*.

The identification of *IId* is based on the following arguments. The mass spectrum shows a base peak at *m/z* 164 from an ion formed through fission of two benzylic bonds in ring C in the molecular ion with ionization of the nonnitrogenous fragment. This fragment confirms the presence[6] of two methoxyl groups in ring D. The lack of an abundant $[M - OCH_3]^+$ fragment excludes CH_3O substitution at C-9, as C-9 substituted tetrahydroprotoberberines are known to exhibit pronounced $[M - OCH_3]^+$ ions in their mass spectra contrary to homologues carrying methoxyl substituents at other positions[6,7]. The relative abundance of the $[M - OCH_3]^+$ ion is only about 1% of the molecular ion. The ^{1}H NMR spectrum (400 MHz, $CDCl_3$) shows a three-proton singlet at δ 2·23 assigned to an acetoxy group confirming the presence of one hydroxyl group in the unacetylated product. Two singlets at δ 3·83 and 3·85, three and six protons, respectively, confirm the presence of three methoxyl groups. The signals of four aromatic protons are all well separated singlets which proves that the protons in ring D cannot be in *ortho* or *meta* positions to each other. These observations indicate that the methoxyl groups are bound to C-10 and C-11. Final proof for the structure of *Id* was obtained from the NOESY and COSY 2D-

NMR spectra of *IId*. The signals from the aliphatic ring protons were easily assigned by combination of the chemical shift information with the information on inter-proton couplings obtained from the COSY spectrum: δ 2·60–2·75 m, 2 H (H-5); 3·10–3·20 m, 2 H (H-6); 3·94 d, 1 H (H-8, J = 14·5); 3·66 d, 1 H (H-8, J = 14·5); 3·58 dd, 1 H (H-14, J = 3·8 and 11·1); 3·21 dd, 1 H (H-13, J = 3·8 and 15·7); 2·84 dd, 1 H (H-13, J = 11·1 and 15·7). The aromatic signals appear at δ 6·94, 6·71, 6·64, and 6·57 and are assigned to H-1, H-4, H-12, and H-9 respectively, based on the following observed NOEs: H-1, H-13, 14; H-4, H-5; H-12, H-13; H-8, H-9. Three aromatic protons, H-4, H-9 and H-12, but not H-1, display NOEs on the methoxyl groups which shows that the acetoxy group is bound to C-2 and the methoxyl groups to C-3, C-10, and C-11. Based on the observed NOEs the six-proton singlet at δ 3·85 is thus assigned to 10-OCH_3 and 11-OCH_3 and the three-proton singlet at δ 3·83 to 3-OCH_3. Thus, the hydroxyl group in *Id* is bound to C-2 and the methoxy groups to C-3, C-10, and C-11 and the protoberberine is pseudocolumbamine. Compound *Id* was isolated by TLC and analysed by ^{1}H NMR spectroscopy. Hydrogenation and acetylation of *Id* gave *IId*.

The fifth component (1%) in the acetylated tetrahydroprotoberberine mixture was not isolated and could not be definitively identified. However, the GC-MS analyses and high resolution mass measurements showed that this component is a tetramethoxy tetrahydroprotoberberine. The mass spectrum reveals that ring A and D are dimethoxy substituted. The relative abundance of the $[M - OCH_3]^+$ ion is low (2% of the molecular ion) which indicates that C-9 is not methoxy substituted.

EXPERIMENTAL

^{1}H NMR spectra were obtained at 400 MHz in CD_3SOCD_3 or $CDCl_3$ with tetramethylsilane as an internal standard. Chemical shifts are given in ppm (δ-scale), coupling constants (J) in Hz. Electron ionization mass spectra (EIMS) (I_E = 70 eV) were recorded using the GC-MS technique. A gas chromatograph equipped with a fused silica column (DB-1, 15 m × 0·53 mm I.D.) and fid was used for gas chromatographic analyses of *IIa*–*IId*. Hydrogen was used as carrier gas (c. 10 ml/min). Column oven temperature was programmed from 250 to 295°C by 10°C/min.

Plant Material

The bark of *Enantia chlorantha* was collected and identified by Dr Denis Ekotto Mengata during 1985 from Douala-Edea Forest Reserve, Cameroon. (D. Ekotto Mengata, Sorep SA., Yaounde Cameroon).

Isolation of Protoberberines

The separation of *Ia*–*Id* was performed by preparative TLC using silica gel 60 F_{254} TLC plates (layer thickness 0·25 mm) and an eluent system consisting of chloroform–methanol–25% ammonia (14 : 4 : 1). The quaternary protoberberines were eluted in the sequence *Ia*, *Ib*, *Ic*, *Id*. The extract was hydrogenated and tetrahydrojatrorrhizine and tetrahydrocolumbamine could be

isolated by preparative TLC by using chloroform–methanol (20 : 1) as solvent. Acetylation followed by gas chromatography at 250–295/10°C/min revealed that *IIb* and *IIc* had almost the same GC retention times and only the sum of them could be determined by GC. Thus the relative amounts of *IIb* and *IIc* were determined from the ^{1}H NMR spectrum of the original extract. The structure of *IIa* was confirmed by comparing spectral data with those of an authentic sample and with published ^{1}H NMR (ref.[8]) and mass spectra[5]. Compound *IId* was isolated by preparative TLC by using chloroform–methanol (20 : 1) as solvent. Hydrogenation reactions were performed by treating the extract with $NaBH_4$ in methanol. Acetylations were performed with neat acetyl chloride.

Palmatine (Ia). ^{1}H NMR (CD_3SOCD_3): 3·24 t, 2 H (H-5, J = 6·2); 3·88 s, 3 H (CH_3O-3); 3·95 s, 3 H (CH_3O-2); 4·08 s, 3 H (CH_3O-10); 4·11 s, 3 H (CH_3O-9); 4·95 t, 2 H (H-6, J = 6·2); 7·11 s, 1 H (H-4); 7·73 s, 1 H (H-1); 8·04 d, 1 H (H-12, J = 9·2); 8·23 d, 1 H (H-11, J = 9·2); 9·06 s, 1 H (H-13); 9·90 s, 1 H (H-8).

Jatrorrhizine (Ib). ^{1}H NMR (CD_3SOCD_3): 3·15 t, 2 H (H-5, J = 6·1); 3·95 s, 3 H (CH_3O-2); 4·08 s, 3 H (CH_3O-10); 4·10 s, 3 H (CH_3O-9); 4·91 t, 2 H (H-6, J = 6·1); 6·85 s, 1 H (H-4); 7·69 s, 1 H (H-1); 8·01 d, 1 H (H-12, J = 9·2); 8·21 d, 1 H (H-11, J = 9·2); 8·97 s, 1 H (H-13); 9·86 s, 1 H (H-8).

Columbamine (Ic). ^{1}H NMR (CD_3SOCD_3): 3·20 t, 2 H (H-5, J = 6·3); 3·90 s, 3 H (CH_3O-3); 4·07 s, 3 H (CH_3O-10); 4·10 s, 3 H (CH_3O-9); 4·93 t, 2 H (H-6, J = 6·3); 7·07 s, 1 H (H-4); 7·57 s, 1 H (H-1); 8·07 d, 1 H (H-12, J = 9·3); 8·20 d, 1 H (H-11, J = 9·3); 8·84 s, 1 H (H-13); 9·88 s, 1 H, (H-8).

Pseudocolumbamine (Id). ^{1}H NMR (CD_3SOCD_3): 3·20 t, 2 H (H-5, J = 6·0); 3·90 s, 3 H (CH_3O-3); 4·00 s, 3 H (CH_3O-11); 4·07 s, 3 H (CH_3O-10); 4·78 t, 2 H (H-6, J = 6·0); 7·08 s, 1 H (H-4); 7·51 s, 1 H (H-1); 7·71 s, 2 H (H-9 and H-12); 8·50 s, 1 H (H-13); 9·88 s, 1 H (H-8).

Tetrahydropalmatine (IIa). EIMS, m/z (rel. int.): 355 $[M]^+$ (100), 354 (61), 340 (10), 324 $[M - OCH_3]^+$ (15), 190 (28), 165 (22), 164 (97), 149 (4).

3-O-Acetyltetrahydrojatrorrhizine (IIb). EIMS, m/z (rel. int.): 383 $[M]^+$ (56), 382 (22), 352 $[M - OCH_3]^+$ (7), 340 (7), 176 (8), 165 (22), 164 (100), 149 (41), 121 (9), 104 (8).

2-O-Acetyltetrahydrocolumbamine (IIc). EIMS, m/z (rel. int.): 383 $[M^+]$ (36), 352 $[M - OCH_3)^+$ (15), 220 (9), 165 (30), 164 (100), 163 (23), 149 (84), 121 (15).

2-O-Acetyltetrahydropseudocolumbamine (IId). EIMS, m/z (rel. int.): 383 $[M]^+$ (24), 382 (7), 165 (29), 164 (100), 149 (3), 121 (7).

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REFERENCES

1. Virtanen P., Teräs M., Lassila V., Njimi T., Ekotto Mengata D.: *Proc. Xth Int. Congr. Pharmacol.*, p. 1013. International Union of Pharmacology, Sydney 1987.
2. Virtanen P., Lassila V., Njimi T., Ekotto Mengata D.: Acta Anat. *132*, 159 (1988).
3. Virtanen P., Lassila V., Njimi T., Ekotto Mengata D.: Acta Anat. *131*, 166 (1988).

4. Ringdahl B., Chan R. P. K., Craig J. C., Manske R. H. F.: J. Nat. Prod. *44*, 75 (1981).
5. Siwon J.: *Thesis*. University of Leiden, Leiden 1982.
6. Ohashi M., Wilson J. M., Budzikiewicz H., Shamma M., Slusarchyk W. A., Djerassi C.: J. Am. Chem. Soc. *85*, 2807 (1963).
7. Richter W. J., Brochmann-Hansen E.: Helv. Chim. Acta *58*, 203 (1975).
8. Ohiri F. C., Verpoorte R., Baerheim Svendsen A.:Planta Med. *49*, 162 (1983).