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STRUCTURE OF KUKOAMINE A, A HYPOTENSIVE PRINCIPLE OF *LYCIUM CHINENSE* ROOT BARKS[1]

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Abstract — From the crude drug "jikoppi", the root barks of *Lycium chinense*, a novel spermine alkaloid, kukoamine A, showing hypotensive activity has been isolated and the structure has been determined as that represented by formula I on the basis of chemical and physical evidence.

The Oriental medicine "jikoppi", the root barks of *Lycium chinense* Miller (Solanaceae), has been shown to be clinically effective for hypertension,[2] and has been reported to exhibit hypotensive, hypoglycemic, antipyretic and anti-stress ulcer activity in experimental animals.[2,3]

Regarding the chemical components of "jikoppi", betaine has been isolated from a Japanese sample,[4] while betaine, a phytosterol and linolic acid have been isolated from a Chinese sample.[4] However, no principle responsible for the above physiological activity has been identified.

Since the methanol extract of a "jikoppi" (0.5 g (crude drug)/kg, *i.v.*) produced significant hypotension in rats, it was repeatedly chromatographed (silica gel/AcOEt and MeOH) by monitoring for hypotensive activity to obtain, together with choline and betaine, an active fraction which on further chromatography (Amberlite XAD-2/H_2O and EtOH, and Sephadex LH-20/MeOH) afforded an amorphous alkaloid, kukoamine A, which induced apparent hypotension in rats (5 mg/kg, *i.v.*).

Kukoamine A was shown to have the composition $C_{28}H_{42}N_4O_6$ (FD-MS (*m/e* 530, M^+)). It gave a positive reaction with Dragendorff's reagent (amines) and ferric chloride (phenols).

Although kukoamine A possesses twenty-eight carbon atoms in the molecule, its ^{13}C NMR spectrum exhibited only fourteen signals, indicating the structure to be symmetric. Accepting that the molecule is symmetric, the ^{13}C NMR spectrum then demonstrates the presence of fourteen aliphatic carbons ($CH_2 \times 14$), twelve aromatic carbons (CH × 6, C × 2, C-O × 4) and two carbonyl carbons.

The presence of two 3,4-dihydroxy-1-alkylbenzene moieties was shown by a positive ferric chloride test, a UV maximum at 284 nm, ^1H NMR signals (each 2H) at δ 6.78 (*J* 8 and 2 Hz), 6.90 (*J* 2 Hz) and 6.99 (*J* 8 Hz) in an ABC pattern which closely resembled that for 4,4'-(2,3-dimethyltetramethylene)-dipyrocatechol,[5] and ^{13}C NMR signals at δ 133.8 (C), 117.0 (CH), 144.6 (C-O), 143.1 (C-O), 117.0 (CH) and 121.6 (CH), whose chemical shifts are consistent with those calculated for $C_{(1)}$-$C_{(6)}$ in a 3,4-dihydroxy-1-alkylbenzene (δ 131.5, 117.9, 142.6, 139.8, 117.1 and 123.3).[6]

Since kukoamine A was thought to have amide groups (a ^{13}C NMR signal at δ 176.9 and an IR band at 1596 cm^{-1}), it was hydrolyzed with hydrochloric acid to afford a product which was separated into a *n*-butanol soluble portion and a water soluble portion.

The butanol soluble portion gave an acid, m.p. 137-139°, $C_9H_{10}O_4$ (MS (*m/e* 182, M^+)) (a UV maximum at 283 nm, IR bands at 3385 (hydroxyl and carbonyl) and 1671 cm^{-1} (carbonyl)) whose NMR spectra showed the presence of a 3,4-dihydroxy-1-alkylbenzene (δ 6.54 (*J* 8 and 2 Hz), 6.67 (*J* 2 Hz) and 6.74 (*J* 8 Hz) along with a CH_2-CH_2 moiety at ca. δ 2.68 for ^1H, and δ 115.6 (CH), 115.8 (CH), 119.9 (CH), 133.2 (C), 143.5 (C-O) and 145.1 (C-O) together with 30.5 (CH_2), 35.8 (CH_2) and

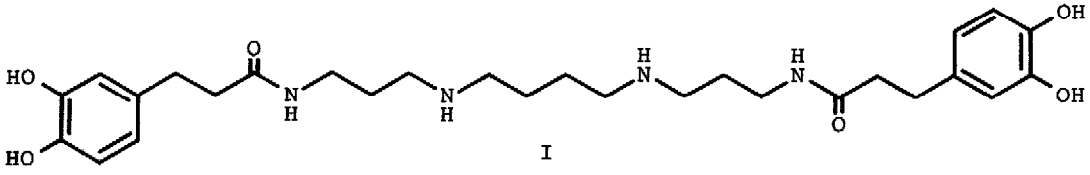
174.2 (COOH) for ^{13}C). The observed chemical shifts of the six ^{13}C NMR signals for the aromatic carbons were compatible with the calculated shifts for $C_{(1)}$-$C_{(6)}$ in a 3,4-dihydroxy-1-alkylbenzene but not with those for a 2,4-dihydroxy-1-alkylbenzene or a 2,5-dihydroxy-1-alkylbenzene. These data showed the acid to be dihydrocaffeic acid which was confirmed by a direct comparison.

As the water soluble portion was shown by TLC analysis to contain two substances, it was chromatographed on Amberlite IRA-400 (OH^- type) to furnish an amide-phenol, which was adsorbed on the resin and eluted with 6N hydrochloric acid, and an amine, which passed through the column.

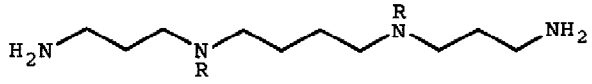
The amine (the tetrahydrochloride, m.p. 296-300°, a MS peak at *m/e* 202 (M^+), ^1H NMR signals at δ 2.15 (8H) and 3.25 (12H), ^{13}C NMR signals at 23.1, 24.1, 37.0, 45.0 and 47.4 (each 2C)) was identified as spermine.

From the accumulated data, it was concluded that kukoamine A can be considered to be made up from two molecules of dihydrocaffeic acid symmetrically combined, by amide linkages, with one molecule of spermine. Two possibilities (I and II) were thus envisaged. Comparison of the ^1H NMR spectrum of kukoamine A with that of maytenine (dicinnamoylspermidine)[7] showed that there are only four hydrogen atoms (two methylenes, δ 3.33) next to nitrogen atoms forming amide linkages.

The structure of kukoamine A was thus determined to be that represented by formula I.



I



II: R=dihydrocaffeoyl

In this connection, the amide-phenol, which was obtained by acid hydrolysis of kukoamine A, is concluded to be the partial hydrolysis product (dihydrocaffeoylspermine), because it has the composition $C_{19}H_{34}O_3N_4$, the ^1H NMR spectrum indicated it to be an amide consisting of one molecule of spermine and one molecule of dihydrocaffeic acid (δ 6.80 (1H), 6.88 (1H) and 6.98 (1H)) and its hydrolysis gave spermine and dihydrocaffeic acid.

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NOTE AND REFERENCES

1) Part 21 in the series on the validity of Oriental medicines.
2) *Zhung jau da ci dian*, p. 819. Shang hai ke hye gi shu chu ban she, Shang hai (1978)
3) J. Yamahara, M. Kim, T. Sawada and H. Fujimura, *Shoyakugaku Zasshi*, 18, 33 (1964)
4) K. Mizobuchi, Y. Inoue, M. Nagai and J. Higashi, *Shoyakugaku Zasshi*, 17, 16 (1963)
5) C. J. Pouchert and J. R. Campbell, *The Aldrich Library of NMR spectra* vol. IV, p. 135. Aldrich Chemical Company, Inc., Milwaukee (1974)
6) G. L. Nelson, G. C. Levy and J. D. Cargioli, *J. Amer. Chem. Soc.*, 94, 3089 (1972)
7) G. Englert, K. Klinga, R. Hamet, E. Schlittler and W. Vetter, *Helv. Chim. Acta*, 56, 474 (1973)

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