85. *The Alkaloids of* Kreysigia multiflora *Reichb. Part I. Isolation.*

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Four alkaloids, kreysigine ($C_{22}H_{27}O_5N$), floramultine ($C_{22}H_{27}O_5N$), kreysiginine ($C_{21}H_{27}O_5N$), and floramultinine ($C_{21}H_{27}O_6N$) have been isolated from *Kreysigia multiflora* Reichb. They were detected by paper chromatography, and separated on cellulose columns. Some of their physical and chemical properties are described.

The genus *Kreysigia*, order Liliaceae, is represented in Australia by only one species, *Kreysigia multiflora* Reichb,* which is a plant growing up to eighteen inches high and having a knotty rhizome.[1]

The dried whole plant † was finely ground and extracted successively with ether and alcohol. A chloroform solution of the combined concentrated extracts was shaken with dilute aqueous hydrochloric acid, and the aqueous extract after several extractions with chloroform was basified with ammonia and re-extracted with chloroform. Evaporation of this extract gave the crude base (0·5%), which showed four main spots on paper chromatography. Separation was achieved by partition on a cellulose column, paper chromatography being used to test homogeneity.

Physical and chemical properties of the alkaloids of Kreysigia multiflora *Reichb.*

Alkaloid	Yield (%)	M. p.	$[\alpha]_D$	Formula	R_F (15°)	Light absorption $\varepsilon_{max.}$ (at mμ)	Light absorption Bands ($cm.^{-1}$)	OMe groups	NMe group	C skeleton
Kreysigine	0·03	188°	Inactive	$C_{22}H_{27}O_5N$	0·55	53,890 (221) 15,600 (260) 6600 (293)	1608, 1577	4	1	C_{17}
Floramultine	0·19	230 (decomp.)	$[\alpha]_D^{18}$ −77° (*c*, 1·19 in $CHCl_3$)	$C_{22}H_{27}O_5N$	0·51	45,100 (220) 13,400 (259) 6500 (287)	3559 * 3448 1597, 1565 †	3	1	C_{18}
Kreysiginine	0·05	149	$[\alpha]_D^{24}$ +89° (*c* 1·98 in EtOH)	$C_{21}H_{27}O_5N$	0·40	36,220 (218) 2240 (274)	3086 * 1613, 1593 † 1510 1667 ‡	3	1	C_{17}
Floramultinine	0·005	165	$[\alpha]_D^{20}$ +118° (*c* 0·169 in EtOH)	$C_{21}H_{27}O_6N$	0·30	13,870 (217) 1960 (275)	3484 * 1603, 1515 † 1634 ‡	2	1	C_{16}

* OH Absorption. † Aromatic C=C. ‡ Aliphatic C=C.

Yields and some physical and chemical properties are listed in the Table. These alkaloids are not identical with any previously described, and it is possible that they represent a new class. From their spectra they appear to be aromatic, and floramultine contains two phenolic groups. Simple mixtures of floramultine and kreysiginine can be separated readily by taking advantage of this property, since kreysiginine is not phenolic. Furthermore, floramultine and diazomethane give dimethoxyfloramultine, so that all the oxygen functions can be accounted for in floramultine by three methoxyl and two phenolic hydroxyl groups. Kreysigine contains four methoxyl groups, but no hydroxyl, as indicated by the infrared spectrum, so that the fifth oxygen atom is probably present as an ether linkage. Kreysiginine contains three methoxyl groups and one alcoholic hydroxyl group

* A preliminary examination of material supplied by Mr. L. J. Webb was made by Messrs. I. J. McCarthy, K. G. Martin, and B. Thompson, working under the direction of Dr. H. J. Rodda; they isolated crude kreysigine and multiflorine.

† This plant material was collected at the Moonpah State Forest and at Coff's Harbour, New South Wales, by Mr. H. C. Hayes of the Forestry Commission of New South Wales, to whom we are greatly indebted.

[1] Bailey, " The Queensland Flora," Pt. V, H. J. Diddams & Co., Brisbane, 1902, p. 1642.

which is apparent from the infrared band at 3086 cm.$^{-1}$ (Nujol), and this is confirmed by determination of active hydrogen. It has a band also at 1667 cm.$^{-1}$, which is probably due to an aliphatic double bond since it disappears on hydrogenation. Both kreysiginine and floramultinine show spots on paper which after being stained with iodine and exposure for some hours to the air become deep orange-coloured, whilst the spots due to kreysigine and floramultine remain a pale yellow. After hydrogenation, kreysiginine shows the typical yellow spot. Floramultinine has two methoxyl groups, but insufficient material precluded more detailed examination. All four alkaloids contain a *N*-methyl group, and kreysigine and kreysiginine form methiodides, indicating that the nitrogen atom is tertiary. A carbon skeleton of 17 is therefore deduced for kreysigine and kreysiginine and of 18 for floramultine and floramultinine.

Experimental

All m. p.s are corrected. Analyses were carried out by the C.S.I.R.O. Microanalytical Service at Melbourne University.

Isolation of the Alkaloids.—The air-dried, finely ground whole plant (4 kg.) of *Kreysigia multiflora* Reichb., collected at Coff's Harbour on February 2—5, 1959, was extracted three times with cold ether, and the combined extracts were concentrated. The plant material was then extracted three times with hot alcohol and the concentrate combined with the ether extract. Previous tests had shown that a further extraction with dilute hydrochloric acid gave only small amounts of base. The combined concentrate was dissolved in chloroform, and this extract shaken with 2·5% aqueous hydrochloric acid (3 × 500 ml.). The separated aqueous acid extract was re-extracted once with chloroform, made alkaline with ammonia solution and extracted with chloroform (6 × 200 ml.). The chloroform extract was dried (Na_2SO_4) and distilled to dryness to give crude alkaloid (22·7 g.). This material when tested by paper chromatography, the solvent being the upper phase obtained by shaking 5% aqueous acetic acid with an equal volume of butanol, and staining with iodine, showed the presence of four spots at R_F values given in the Table. Attempts were first made to separate the bases on alumina, and although kreysigine could be obtained pure in this way a complete separation of the other bases was not achieved and a considerable amount of floramultine was lost due to its phenolic properties. A cellulose column proved satisfactory provided that the mixed fractions were recycled. A column (83 × 5 cm.) holding about 750 g. of powdered cellulose was packed by tamping with a perforated plunger, butanol–acetic acid–water being used as solvent. The crude base (22·7 g.) was dissolved in the minimum of solvent and put on the top of the column, followed by more solvent. Fractions (50 ml.) were collected automatically,[2] and tested for homogeneity by paper chromatography. The pure fractions were combined separately, whilst the mixed fractions were put through the same column again. Four such columns separated all but 4·8 g. of a mixture of floramultine and kreysiginine, and another fraction of crude floramultinine. The mixture of floramultine and kreysiginine (4·8 g.) was warmed with 10% aqueous potassium hydroxide (20 ml.), and extracted with chloroform (3 × 20 ml.). The combined chloroform extracts were dried and distilled to give pure kreysiginine (0·96 g.), as shown by paper chromatography and recrystallisation (m. p. 149·5°). The aqueous solution was then treated with aqueous (1 : 5) hydrochloric acid (14 ml.); a solid separated. The solution was made strongly alkaline with ammonia solution and extracted with chloroform. The dried chloroform extract gave pure floramultine (2·7 g.).

The crude floramultinine fraction was passed through a cellulose column (48 × 3·5 cm.); fractions (25 ml.) were collected and again tested for purity by paper chromatography. Pure floramultinine (0·16 g.) was obtained.

Kreysigine.—*Kreysigine* had m. p. 187—188°, from alcohol (Found: C, 68·4; H, 7·0; N, 4·2; O, 21·0; OMe, 32·1; *N*-Me, 3·4%; *M* (Rast), 381. $C_{22}H_{27}O_5N$ requires C, 68·5; H, 7·1; N, 3·6; O, 20·8; 4OMe, 32·2; *N*-Me, 3·9%; *M*, 385). This compound is optically inactive, and insoluble in aqueous sodium hydroxide; it gives a dark red colour with concentrated nitric acid and a faint green with ferric chloride. The ultraviolet and infrared data are shown in Table 1.

[2] Box and Bradbury, *J. Sci. Instr.*, 1957, **34**, 183.

Kreysigine Methiodide.—Kreysigine (0·33 g.), methanol (5 ml.), and methyl iodide (10 ml.) were refluxed for 1 hr., and the excess of solvent and methyl iodide removed. The *product*, when recrystallised from alcohol, gave prisms, m. p. 265—266° (Found: C, 52·2; H, 5·8; N, 2·6; O, 15·6. $C_{22}H_{27}O_5N,CH_3I$ requires C, 52·4; H, 5·7; N, 2·7; O, 15·2%).

Floramultine.—From alcohol or benzene it had m. p. 230° (decomp.), $[\alpha]_D^{18}$ −77° (*c* 1·19 in chloroform) [Found: C, 68·5; H, 6·9; N, 3·6; O, 21·4; OMe, 24·5; *N*-Me, 3·3%; Equiv. (by titration), 398. $C_{22}H_{27}O_5N$ requires C, 68·5; H, 7·1; N, 3·6; O, 20·8; 3OMe, 24·2; *N*-Me, 3·9%; *M*, 385].

Dimethoxyfloramultine.—A solution of floramultine (0·4 g.) in methanol (25 ml.) was added to a solution of diazomethane in ether [from methylnitrosourea (10·3 g.)] and kept for 16 hr. The solution was taken to dryness; attempts to recrystallise the product from alcohol and benzene–light petroleum failed even after further chromatography. The *hydrobromide*, prepared by exact neutralisation with 0·1N-hydrobromic acid and evaporation of the solution, had m. p. 243° (decomp.) from alcohol (Found: C, 56·3; H, 6·3; OMe, 32·0. $C_{24}H_{31}O_5N,HBr,H_2O$ requires C, 56·2; H, 6·7; 5OMe, 30·3%). The water of crystallisation could not be completely removed at 116° in a high vacuum.

Kreysiginine.—This was obtained as large prisms (from ether or from a concentrated alcohol solution), m. p. 149°, $[\alpha]_D^{24}$ +89° (*c* 1·98 in ethanol) [Found: C, 67·6; H, 7·4; N, 3·7; O, 21·4; OMe, 24·7; *N*-Me, 3·6; *C*-Me, nil; active H, 0·19%; Equiv. (by titration), 375; *M* (Rast), 373. $C_{21}H_{27}O_5N$ requires C, 67·5; H, 7·3; N, 3·7; O, 21·4; 3OMe, 24·9; *N*-Me, 4·02; 1 active H, 0·27%; *M*, 373].

Kreysiginine Methiodide.—Kreysiginine (0·25 g.), methanol (5 ml.), and methyl iodide (10 ml.) were refluxed for 1 hr. The solvent was removed and the residue recrystallised from acetone. Prisms softening at 150°, with swelling but showing no true melting point, were obtained [Found: C, 52·3; H, 6·4; N, 2·2; O, 16·9; I, 22·1; *C*-Me, 1·7. $C_{21}H_{27}O_5N,CH_3I,(CH_3)_2CO$ requires C, 52·3; H, 6·3; N, 2·4; O, 16·8; I, 22·1; 1*C*-Me, 2·6%].

The infrared spectrum of this compound showed a carbonyl band at 1704 cm.$^{-1}$ which was not present in kreysiginine; this confirms the presence of acetone of crystallisation.

Kreysiginine Hydrobromide.—The alkaloid (0·102 g.) was dissolved in hot ethanol (5 ml.), and titrated with 0·1N-hydrobromic acid to the Bromocresol Green end-point. The solution was evaporated, extracted with ether, and then evaporated to dryness. The dry residue was dissolved in alcohol, the solution poured into ether, and the precipitated *hydrobromide* dried at 78° *in vacuo*; it had m. p. 142—143° (Found: C, 53·1; H, 6·5; N, 3·0. $C_{21}H_{27}O_5N,HBr,H_2O$ requires C, 53·4; H, 6·4; N, 3·0%).

Hydrogenation of Kreysiginine.—Kreysiginine (0·102 g.), platinum oxide (0·1 g.), and acetic acid (10 ml.) were shaken in the presence of hydrogen for 5 hr., and then kept for 18 hr.; approximately 2 mol. of hydrogen were absorbed. The product, after the removal of the platinum and the solvent, was an oil, $[\alpha]_D^{23}$ +59° (*c* 0·20 in ethanol). The ultraviolet spectrum showed maxima at 218 and 285 mμ, the band at 275 mμ in kreysiginine being absent. The band at 1669 cm.$^{-1}$ is also absent. It is notable also that the spots obtained on paper after staining with iodine did not become orange-coloured but remained the normal yellow.

Attempts to prepare a perchlorate were unsuccessful, but a *hydrobromide* was prepared as described above (Found: C, 52·2; H, 7·0; N, 2·9. $C_{21}H_{31}O_5N,HBr,H_2O$ requires C, 52·9; H, 7·2; N, 2·9%).

Floramultinine.—This *compound* was soluble in water. It was purified by precipitation from benzene with light petroleum, and had m. p. 165°, $[\alpha]_D^{20}$ +118° (*c* 0·17 in ethanol) (Found: C, 64·5; H, 7·1; N, 3·8; OMe, 16·7; *N*-Me, 3·1. $C_{21}H_{27}O_6N$ requires C, 64·8; H, 7·0; N, 3·6; 2OMe, 15·9; *N*-Me, 3·1%).

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