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METHANOL-SOLUBLE QUATERNARY ALKALOIDS FROM AFRICAN *FAGARA* SPECIES*

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Key Word Index—*Fagara*; Rutaceae; chemotaxonomy; quaternary alkaloids; candicine; magnoflorine; tembetarine.

Abstract—The quaternary alkaloids from several African species of *Fagara* have been examined by TLC and high voltage electrophoresis. All species investigated have been found to contain a complex mixture of similar quaternary bases derived via the phenylalanine/benzylisoquinoline biosynthetic pathway. Magnoflorine has been isolated from *F. leprieurii*, *F. rubescens* and *F. viridis*, while *F. chalybea* has yielded tembetarine and candicine. The chemosystematic potential of these and previously reported chloroform-soluble bases is considered.

INTRODUCTION

THE GENUS *Fagara* L. (Rutaceae), of pan-tropical distribution, is represented in Africa by about forty species. According to Engler[1] these are all grouped in the Gerontogaeae Engl. as part of the large sub-section Paniculatae Engl. within the Section Macqueria Triana et Planch. The taxonomy of the genus and its relationship with the closely allied *Zanthoxylum* L. is confused. As part of a programme of research into the chemosystematics of the group we have recently examined seven *Fagara* species of African origin.

Previously isolated alkaloids[2–17] from those species (Table 1) are all derived through either the anthranilic acid or phenylalanine/benzylisoquinoline metabolic pathways. The most common alkaloids have been found to be the furoquinoline skimmianine and the quaternary benzophenanthridines chelerythrine and nitidine. In addition, all bark samples

* Part I in a projected series "Chemosystematics in the Rutaceae".

[1] A. ENGLER, in *Die Naturliche Pflanzen-familien* (edited by A. ENGLER and K. PRANTL), 2nd Edition, Vol. 19a, p. 217, Engelmann, Leipzig (1931).
[2] K. H. PALMER, Ph.D. Thesis, University of Paris (1956).
[3] L. FONZES and F. WINTERNITZ, *Compt. Rend.* **266,** 930 (1968).
[4] L. FONZES and F. WINTERNITZ, *Phytochem.* **7,** 1889 (1968).
[5] F. FISH and P. G. WATERMAN, *Phytochem.* **10,** 3322 (1971).
[6] F. FISH and P. G. WATERMAN, *J. Pharm. Pharmac.* **23,** 132S (1971).
[7] H. THOMS and F. THUMEN, *Chem. Ber.* **44,** 3717 (1911).
[8] R. PARIS and H. MOYSE-MIGNON, *Ann. Pharm. France* **5,** 410 (1947).
[9] D. A. H. TAYLOR and I. T. ESHIETT, *J. Chem. Soc.* 481 (1968).
[10] F. G. TORTO, P. SEFCOVIC and B. A. DADSON, *Tetrahedron Letters* 181 (1966).
[11] J. A. GOODSON, *Biochem. J.* **15,** 123 (1921).
[12] F. G. TORTO, P. SEFCOVIC, B. A. DADSON and I. A. MENSAH, *Ghana J. Sci.* **9,** 3 (1969).
[13] F. G. TORTO and I. A. MENSAH, *Phytochem.* **9,** 911 (1970).
[14] J. M. CALDERWOOD, N. FINKLESTEIN and F. FISH, *Phytochem.* **9,** 675 (1970).
[15] J. M. CALDERWOOD, N. FINKLESTEIN, F. FISH and R. T. PARFITT, *Phytochem.* **10,** 682 (1971).
[16] F. FISH and P. G. WATERMAN, *Phytochem.* **11,** 1866 (1972).
[17] F. FISH and P. G. WATERMAN, *Phytochem.* **10,** 3325 (1971).

so far investigated have been found to contain considerable quantities of quaternary alkaloids in the methanol extracts.[18] With the exception of the chloroform-soluble chelerythrine, nitidine and *N*-methyltetrahydropalmatine, the identification of the quaternary alkaloids had not been verified.

TABLE 1. ALKALOIDS ISOLATED PREVIOUSLY FROM AFRICAN *Fagara* SPECIES

Species	Alkaloids	Ref.
Fagara	Skimmianine (I)	2
leprieurii	1-Hydroxy-2,3-dimethoxy-10-methyl-acridan-9-one (II)	3
(Guill. et Perr.) Engl.	Chelerythrine (VII)	4
	Nitidine (VIII)	5
	1-Hydroxy-3-methoxy-10-methyl-acridan-9-one (III)	
F. rubescens	Skimmianine	6
(Planch. ex Hook. f.) Engl.	Chelerythrine	
	Nitidine	
	1-Hydroxy-2,3-dimethoxy-10-methyl-acridan-9-one	
	1-Hydroxy-3-methoxy-10-methyl-acridan-9-one	
F. xanthoxyloides Lam.	Fagaramide (VI)	7
	Skimmianine	8, 9
	3-Dimethylallyl-4-methoxy-2-quinolone (V)	9
	Chelerythrine	10
F. macrophylla	Fagaramide	11
(Planch. ex Oliv.) Engl.	Skimmianine	12
	Chelerythrine	
	Nitidine	13
F. capensis Thunb.	Skimmianine	14
	Chelerythrine	
	Nitidine	
	N-Methyltetrahydropalmatine (XIX)	15
F. chalybea Engl.	Skimmianine	16
	Chelerythrine	
	Nitidine	
F. viridis A. Chev.	Canthin-6-one (IV)	17
	Chelerythrine	
	Nitidine	

Since, in some cases, the quantity of material available was insufficient for the isolation and full characterization of the individual constituents, these were identified by means of high voltage electrophoresis and TLC (3 systems) using methods previously described.[19] Partially purified extracts were examined with the following reference compounds: the phenylethylamines candicine (IX) and coryneine (X), the benzylisoquinoline tembetarine (XI), the 1,2,10,11-tetrasubstituted aporphines magnoflorine (XII), *N*-methylcorydine (XIII) and *N*-methylisocorydine (XIV), the 1,2,9,10-tetrasubstituted aporphines xanthoplanine (XV) and laurifoline (XVI), berberine (XVII), and the tetrahydroprotoberberine *N*-methylcanadine (XVIII). Magnoflorine, tembetarine and candicine were subsequently isolated in sufficient yields from certain species to allow full characterization.

RESULTS

The tentative identification of quaternary alkaloids in the African species, based on chromatographic and electrophoretic techniques, is given in Table 2. With the systems

[18] J. M. CALDERWOOD and F. FISH, *J. Pharm. Pharmac.* **18,** 119S (1966).
[19] J. M. CALDERWOOD and F. FISH, *J. Pharm. Pharmac.* **21,** 126S (1969).

available, identification of most bases IX-XVIII was achieved, the exception being *N*-methylcorydine (XIII) and *N*-methylisocorydine (XIV), between which no positive distinction was possible.

TABLE 2. METHANOL-SOLUBLE ALKALOIDS IDENTIFIED FROM AFRICAN *Fagara* SPECIES

Species	Voucher No.* Root bark	Stem bark‡	Source	IX	X	XI	XII	XIII–XIV	XV	XVI	XVII	XVIII§
Fagara leprieurii	FF 1	FF 2	T.P.I., Nigeria	tr	—	++	+++	+	—	—	—	—
	FF 3	FF 4	T.P.I., Nigeria	—	—	++	+++	+	—	—	—	—
		FF 25	Kenya Govt.	+	—	++	+++	++	—	—	—	—
F. rubescens	FF 5	FF 6	T.P.I., Nigeria	++	—	++	++++	+	—	—	—	—
F. viridis	FF 8	FF 9	T.P.I., Nigeria	tr	—	+	+++	+	—	—	—	—
	FF 10	FF 11	Hardman, Nigeria	—	—	+	++	+	—	—	—	—
F. chalybea	FF 12	FF 13	T.P.I., Kenya	+++	—	+++	+	+	—	—	+	—
	Gibson 183 (SRGH)		Raffingora, Rhodesia	++	—	++	+	tr	—	—	tr	—
F. macrophylla	FF 16	FF 17	T.P.I., Nigeria	—	—	+	+	+	—	—	+	—
	FF 18	FF 19	T.P.I., Nigeria	—	—	tr	++	+	—	—	+	—
	A.A.Enti Sp. 391(E)		Esen ne Pam Forest Reserve, Ghana	—	—	+	+	++		—	+	—
F. xanthoxyloides	FHI, 56532		T.P.I., Nigeria	—	—	+	++	tr	—	—	tr	—
	FF 27	FF 28	M'Bao Forest, Dakar, Senegal	—	—	+	+	+	—	—	+	—
	A.A.Enti Sp. 159(E)		Nuanga, Agric. Research Station, Ghana	—	—	tr	+	++	—	—	tr	—
F. capensis	FF 20	FF 21	South Africa	tr	—	+	++	+++	—	—	+	—
	H. Schutte 72(PRE)†		Transvaal, South Africa	+	—	+	+++	++	—	—	tr	—

* For specimens in the FF series, voucher samples have been deposited with the herbarium of the Pharmaceutical Society at the University of Bradford, England. The other reference specimens are in the herbaria indicated (see *Index Herbariorum*).

† As *F. magalismontana* Engl.

‡ In all cases where root and stem barks were examined, the alkaloidal patterns were the same in both, although root barks were relatively richer than stem barks.

§ Key: IX, candicine; X, coryneine; XI, tembetarine; XII, magnoflorine; XIII, *N*-methylcorydine; XIV, *N*-methylisocorydine; XV, xanthoplanine; XVI, laurifoline; XVII, berberine; XVIII, *N*-methylcanadine.

In all species investigated magnoflorine was indicated together with smaller quantities of one or both of its monomethyl ethers; with the exception of *F. macrophylla* and *F. chalybea* it appears to be the major quaternary base. Its isolation from *F. leprieurii*, *F. rubescens* and *F. viridis* was achieved by repeated recrystallisation of the precipitated magnoflorine iodide from dry methanol. The structure was elucidated by preparation of the styphnate, comparison with published data on ORD and UV spectra,[20] and interpretation of the mass spectrum (to be published), details of which related well with known MS data on the tertiary aporphines.[21]

Two further alkaloids were isolated from *F. chalybea* by fractional precipitation from dry methanol using ethyl acetate and diethyl ether. One of them was identified as candicine by direct comparison with an authentic sample synthesized from hordenine.[22] The second was identified as the quaternary benzylisoquinoline tembetarine by the preparation of the

[20] S. M. ALBONICO, J. COMIN, A. M. KUCK, E. SANCHEZ, P. M. SCOPES, R. J. SWAN and M. J. VERNENGO, *J. Chem. Soc.* **C**, 1340 (1966).

[21] A. OHASHI, J. M. WILSON, H. BUDZICKIEWICZ, M. SHAMMA, W. A. SLUSARCHYK and C. DJERASSI, *J. Amer Chem. Soc.* **85**, 2807 (1963).

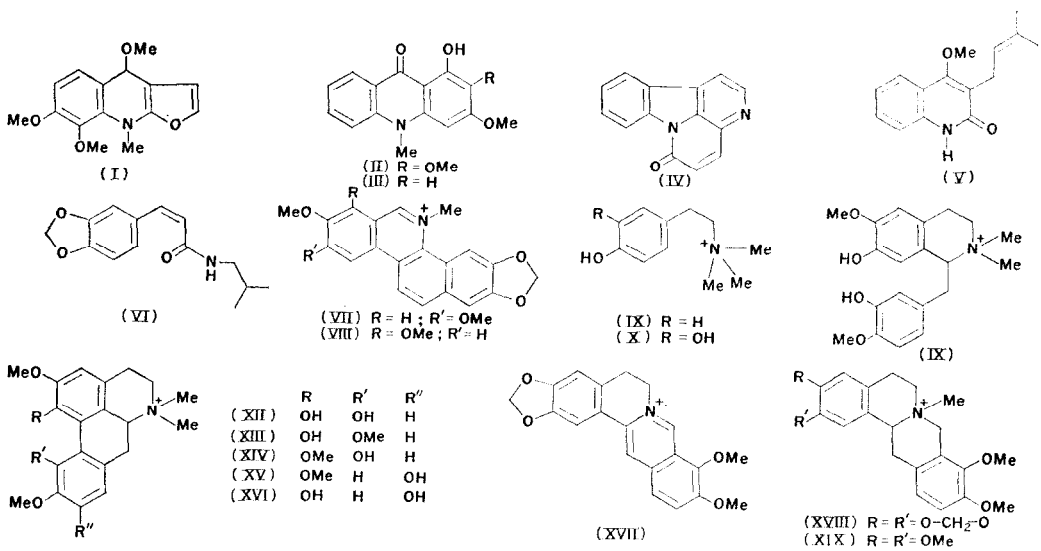
[22] T. NAKANO, *Pharm. Bull. Japan* **2**, 321 (1954).

chloride and styphnate. The UV spectrum[20] and PMR (D_2O) spectrum[23] were in agreement with published data and the MS was in general agreement with reported spectra of 1-benzyl-1,2,3,4-tetrahydroisoquinolines.[21]

Very small quantities of an alkaloid were isolated from *F. macrophylla*. Spectral evidence (UV, MS) indicated that it probably possesses a protoberberine nucleus[21] but no firm structure can be suggested for this alkaloid.

DISCUSSION

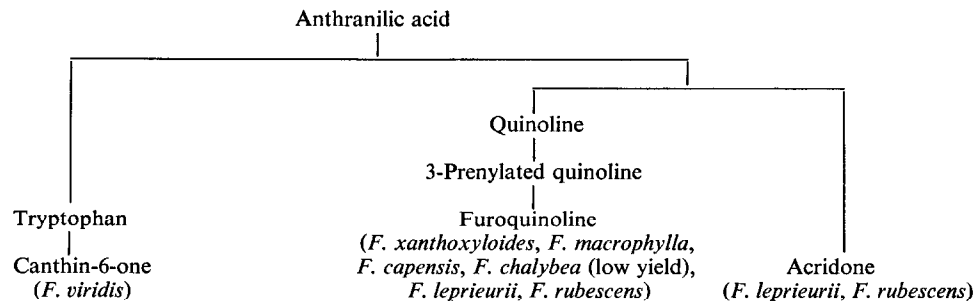
The similarity of alkaloid patterns in all seven species examined is striking, the products of anthranilic acid (I–V) and phenylalanine/benzylisoquinoline (VI–XIX) metabolism (both pathways being of shikimic acid origin) occurring without exception.



With respect to those bases derived from anthranilic acid, the common occurrence of the furoquinoline skimmianine is the most marked feature. The furoquinoline nucleus can be regarded as characteristic of the Rutaceae having been reported from the vast majority of genera investigated. The replacement of skimmianine by a consistently high yield of canthin-6-one is an interesting diversification noted here in *F. viridis* and previously reported in *F. ovalifolia* (Wight) Engl. (syn. *Zanthoxylum suberosum* C. T. White, *Z. dominianum* Merr. and Perr.),[24,25] *F. caribea* (Lam.) Krug. et Urb.[26] and *F. elephantiasis* (Macf.) Krug. et Urb.[27] None of those species has been reported to contain skimmianine or any other anthranilic acid-derived alkaloid. It is interesting to speculate on the biosynthesis of canthin-6-one; anthranilic acid is considered to be the precursor of tryptophan[28] and it has been suggested that canthin-6-one may be formed by the direct combination of

[23] S. M. ALBONICO, A. M. KUCK and V. DEULOFEU, *Annalen* **685**, 200 (1965).
[24] J. R. CANNON, G. K. HUGHES, E. RITCHIE and W. C. TAYLOR, *Austral. J. Chem.* **6**, 86 (1953).
[25] G. B. GUISE, E. RITCHIE, R. G. SENIOR and W. C. TAYLOR, *Austral. J. Chem.* **20**, 2429 (1967).
[26] D. D. CASA and M. C. SOJO, *J. Chem. Soc.* **C**, 2155 (1967).
[27] A. T. AWAD, J. L. BEAL, S. K. TALAPATRA and M. P. CAVA, *J. Pharm. Sci.* **56**, 279 (1967).
[28] D. GROGER, *Lloydia* **32**, 221 (1969).

tryptophan with a derivative of aspartic acid[29] or glutamic acid.[30] It would seem therefore that *F. viridis* and the canthin-6-one producing taxa have the facility to combine anthranilic acid with other amino acids to form first tryptophan and then canthin-6-one, whilst in the majority of *Fagara* taxa the alternative combination of anthranilic acid and acetate to yield the quinoline nucleus occurs.[31] The possibility that furoquinolines may be derived by degradation of tryptophan has now been discounted by tracer experiments.[32] The isolation of 3-dimethylallyl-4-methoxy-2-quinolone, a precursor of the furoquinoline ring system, from the heartwood of *F. xanthoxyloides* is of interest with regard to the site of biosynthesis of the furoquinoline nucleus but has little chemosystematic significance. The co-occurrence of the furoquinoline and acridone nuclei in *F. leprieurii* and *F. rubescens* indicates a new facet of anthranilic acid metabolism in *Fagara* since, although the acridones are common constituents of several other genera of Rutaceae, they have not yet been reported elsewhere in *Fagara*. They are considered to be formed from anthranilic acid with the addition of three acetate units.[33] The inter-relationship of the anthranilic acid-derived alkaloids of the seven African species is demonstrated in Scheme I.

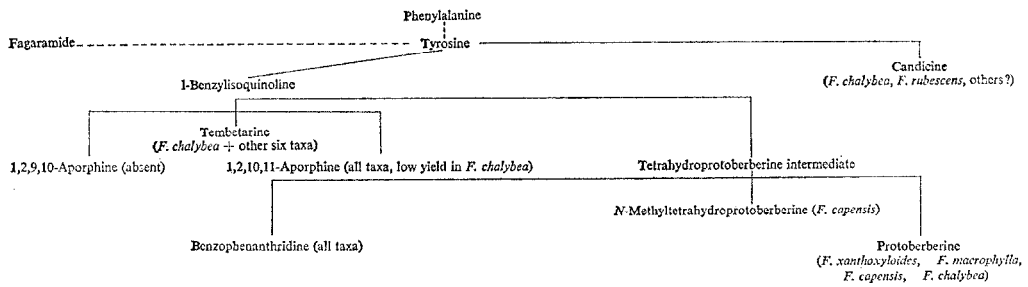


SCHEME 1. SUGGESTED BIOGENESIS OF *Fagara* ALKALOIDS DERIVED FROM ANTHRANILIC ACID.

With regard to phenylalanine/benzylisoquinoline metabolism, two pathways occur in all seven species, leading to the aporphine and benzophenanthridine nuclei both of which require a 1-benzylisoquinoline intermediate. It is noteworthy that only 1,2,10,11-substituted aporphines are present, no trace of the 1,2,9,10-substituted laurifoline or xanthoplanine having been detected. It would appear that all the African species investigated have the ability to form the aporphine ring system by oxidative coupling *ortho*, but not *para*, to the hydroxy group of the 1-benzyl substituent of the benzylisoquinoline precursor. With regard to the benzophenanthridines, chelerythrine predominates in six of the taxa, the exception being *F. macrophylla* where nitidine appears to be the major alkaloid. Significantly, *ortho* coupling would also be required in the production of the tetrahydroprotoberberine intermediate leading to chelerythrine, whilst *para* coupling leads to the less abundant nitidine. Variations in the ability to couple *ortho* or *para* has been demonstrated in South American taxa and the chemosystematic potential of this has been discussed.[34]

[29] R. ROBINSON, *The Structural Relations of Natural Products*, p. 111, Clarendon Press, Oxford (1955).
[30] E. LEETE, in *Biogenesis of Natural Compounds* (edited by P. BERNFIELD), 2nd Edition, p. 990, Pergamon Press, Oxford (1967).
[31] I. MONKOVIC and I. D. SPENSER, *Chem. Commun.* 204 (1966).
[32] I. MONKOVIC, I. D. SPENSER and A. O. PLUNKETT, *Can. J. Chem.* **45**, 1935 (1967).
[33] I. H. BOWEN, P. GUPTA and J. R. LEWIS, *Chem. Commun.* 1625 (1967).
[34] A. M. KUCK, S. M. ALBONICO, V. DEULOFEU and M. G. ESCALANTE, *Phytochem.* **6**, 1541 (1967).

In six of the seven species investigated by us the two benzylisoquinoline pathways appear to be equally prolific. In the seventh, *F. chalybea*, the benzophenanthridine pathway predominates at the expense not only of the aporphines but also with regard to the furoquinolines. *Fagara chalybea* is further distinguished by the production of considerable quantities of tembetarine (*N*-methylreticuline) and candicine. The former could also be detected in the other taxa but in smaller concentration. The position of tembetarine in the biosynthetic pathway is at present obscure: it has proved to be an inefficient precursor for oxidative coupling in the Papavaraceae[35] but no work has been carried out in the Rutaceae. Because of its reported inefficiency, and also its occurrence in detectable amounts in all taxa, it would seem more likely that reticuline is the true intermediate and that tembetarine is simply another example of the ubiquitous *N*-methyl quaternisation of the *Fagara/Zanthoxylum* complex. The TLC identification of berberine, together with tembetarine, in four of the species is in conflict with the hypothesis that tembetarine and the protoberberines are alternative pathways and that the presence of one leads to the absence of the other.[36] Candicine, the remaining alkaloid of *F. chalybea*, is an early product of the phenylalanine pathway, presumably formed by quaternisation of tyramine. It is also found to a lesser extent in *F. rubescens* and in barely detectable concentrations in some other species. Similarly, in *F. capensis* *N*-methyltetrahydropalmatine would be formed by quaternisation of the tetrahydroprotoberberine intermediate whilst the previously mentioned berberine indicates oxidation leading to a non-methylated quaternary. Again both end products are the result of *ortho* coupling. The suggested biogenetic pathway of the phenylalanine/benzylisoquinoline alkaloids is given in Scheme 2.



SCHEME 2. SUGGESTED BIOGENESIS OF *Fagara* ALKALOIDS DERIVED FROM PHENYLALANINE/BENZYLISOQUINOLINE.

A further point of interest is raised by the isolation of fagaramide from *F. macrophylla* and *F. xanthoxyloides*. This amide has also recently been reported from the fruit of *Fagara laurentii* De Wild.[37] Fagaramide may well be derived by an early modification of phenylalanine or a derivative involving an initial loss of nitrogen, this logically being followed by condensation with an amino acid (probably valine) to yield the amide. This points to a close relationship between *F. macrophylla* and *F. xanthoxyloides* which is confirmed by an

[35] D. H. R. BARTON, R. B. BOAR, D. A. WIDDOWSON, V. DEULOFEU and S. M. ALBONICO, *J. Chem. Soc.* **C**, 807 (1969).
[36] A. R. SKERL and E. G. GROS, *Phytochem.* **10**, 2719 (1971).
[37] M. PARIS and G. ISKANDER, *Plant. Med. Phytother.* **4**, 150 (1970).

examination of their other alkaloids. In this context the isolation of the remaining alkaloids of *F. laurentii* is awaited with interest.

In summary, it appears that the seven African species can be grouped chemotaxonomically on the basis of their alkaloids as follows: (a) *F. viridis*; (b) *F. leprieurii*, *F. rubescens*; (c) (i) *F. macrophylla*, *F. xanthoxyloides* (*F. laurentii*); (ii) *F. capensis*; (iii) *F. chalybea*. Looking first at the anthranilate pathway, *F. viridis* may at once be separated due to the presence of canthin-6-one. Within the furoquinoline-producing taxa, *F. leprieurii* and *F. rubescens* are distinguished by their additional production of acridone alkaloids leaving the other four taxa undivided. With regard to phenylalanine/benzylisoquinoline metabolism, *F. viridis*, *F. leprieurii* and *F. rubescens* are very similar. Of the remainder, *F. xanthoxyloides* and *F. macrophylla* are similar but there is the apparent predominance of *para* coupling in the formation of nitidine in *F. macrophylla*.

Fagara capensis is the only taxon so far reported to contain an *N*-methyltetrahydroprotoberberine while *F. chalybea* appears to differ qualitatively in the production of large quantities of phenylethylamine, benzylisoquinoline and benzophenanthridine at the expense of the usual aporphines and furoquinolines. Finally, the presence of berberine in the four species comprising the skimmianine-only group (c) and its absence from the canthin-6-one and acridone groups supports the initial grouping.

EXPERIMENTAL

Extraction. The ground root bark and stem bark (scraped to remove adhering epiphytes) had previously been extracted with light petrol. (b.p. 40–60°) and $CHCl_3$ to remove all tertiary and $CHCl_3$-soluble quaternary alkaloids. The barks were then extracted with MeOH to exhaustion and the extracts concentrated under reduced pressure. Partial purification was achieved using methods described previously.[6]

Isolation. Magnoflorine. The partially purified extracts from *Fagara leprieurii*, *F. rubescens* and *F. viridis* were evaporated to dryness under reduced pressure and dissolved in the minimum vol. of distilled H_2O. A saturated solution of KI was added dropwise until no further precipitation occurred. Repeated recrystallization from dry MeOH yielded white prisms of magnoflorine iodide ($C_{20}H_{24}NO_4^+I^-$) m.p. 247–51° (lit.[38] m.p. 249°) UV λ_{max}^{EtOH} 271, 310 (log ϵ 3·92, 3·81) in agreement with that previously reported.[20] PMR (TFA) indicated the presence of two methoxyl and two *N*-methyl groups, whilst the absence of any low field aromatic proton confirmed the 1,11-substitution.[39] The identity of magnoflorine was confirmed by the preparation of the styphnate m.p. 233° (EtOH) (lit.[38] m.p. 233°). MS did not yield a parent ion but gave P^+-1 341·1611; $C_{20}H_{23}NO_4$ required 341·1627. Methylation (CH_2N_2) gave *O*-dimethylmagnoflorine yielding a P^+-1 ion 28 m.u. in excess of magnoflorine.

Alkaloids of Fagara chalybea. The combined root bark and stem bark extracts of *F. chalybea* were evaporated under reduced pressure to yield an oil. This was dissolved in a minimum volume of EtOH, and dry EtOAc added dropwise until a precipitate formed. The precipitate was separated, redissolved in absolute alcohol and reprecipitated in this manner several times. Recrystallization twice from EtOH–EtOAc (17:3) yielded crystals of *tembetarine chloride*, m.p. 236° (lit.[23] 237°). UV λ_{max}^{EtOH} 286 (log ϵ 3·82). The identity of tembetarine was confirmed by the preparation of the styphnate m.p. 180° (lit.[39] 177–178°). MS did not yield a parent ion but gave P^+-1 343·1772; $C_{20}H_{25}NO_4$ requires 343·1783. The bulked supernatant solutions were evaporated to dryness, dissolved in a minimum vol. of distilled H_2O and precipitated by the dropwise addition of a satd soln of KI. On recrystallization from EtOH the precipitate yielded prisms of *candicine iodide* m.p. 229° (lit.[22] 229–230°) identical with an authentic sample of candicine iodide (m.m.p., UV, IR, PMR, TLC).

Alkaloid from Fagara macrophylla. An attempt to isolate magnoflorine as the iodide from *F. macrophylla* gave instead a precipitate which on recrystallization from dry MeOH yielded brown crystals (pure by TLC) m.p. 197° UV λ_{max}^{EtOH} 264, 342, 410 (indicative of the protoberberine nucleus).[40]

[38] T. KAMETANI, *The Chemistry of the Isoquinoline Alkaloids*, p. 95, Elsevier, London (1969).
[39] K. G. PACHLER, R. R. ARNDT and W. H. BAARSCHERS, *Tetrahedron* **21**, 2159 (1965).
[40] A. W. SANGSTER and K. L. STUART, *Chem. Rev.* **65**, 69 (1965).

M.ps (uncorrected), were determined on a Kofler hot-stage, UV spectra were recorded in EtOH and IR spectra in KCl, PMR spectra (60 MHz) were recorded on a Perkin–Elmer R12 instrument with TMS or DSS as internal standard. MS were determined on a double-focusing AEI MS902 spectrometer.

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