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Screening for tertiary and quaternary alkaloids in some African *Fagara* species

J. M. CALDERWOOD AND F. FISH

A method has been described for the separate extraction of tertiary and quaternary alkaloids from plant material. Different thin-layer chromatographic systems have been used to separate compounds within each group of alkaloids. The methods have been applied to the screening of the barks of five *Fagara* species in which the presence of angoline, angolinine, skimmianine and, in some cases, 1-hydroxy-2,9,10-trimethoxy-*NN*-dimethylaporphinium chloride is indicated.

IN recent research on alkaloids of the family Rutaceae interest has attached to the genus *Fagara* because of an attempt to differentiate it chemotaxonomically from the closely related *Zanthoxylum* (Price, 1963). Failure to clarify the taxonomic position was largely the result of inadequate information on the types of alkaloids present in both genera. The botanical classification of many of the plants investigated is confused, particularly of the Asiatic species some of which Engler (Engler & Prantl, 1931) transferred from the *Zanthoxylum* to the *Fagara*; this transfer has not been recognised in much of the subsequent chemical literature. The genus *Fagara* contains approximately 250 species several of which have been reported to contain alkaloids (Table 1).

In the present work barks of the African species *F. leprieurii* Engl., *F. macrophylla* Engl., *F. viridis* A. Cheval. and *F. xanthoxyloides* Lam., all previously known to contain tertiary alkaloids (Table 2), and of *F. chalybea* Engl., not previously investigated, were examined for both tertiary and quaternary alkaloids.

Experimental

MATERIALS

The stem and root barks of *F. chalybea* Engl. were collected in Kenya, and those of *F. leprieurii* Engl., *F. macrophylla* Engl., *F. viridis* A. Cheval., and *F. xanthoxyloides* Lam. were collected in Nigeria: all were authenticated at source and supplied by The Tropical Products Institute, London.

Alumina for thin-layer chromatography (Camag). The plates (0·25 mm) were activated (3 hr) at 120° and stored in a desiccator for not more than five days.

Cellulose for thin-layer chromatography (Whatman Chromedia CC 41). The plates (0·25 mm) were stored in the absence of a desiccant.

Chloroform (ethanol-free and dried).

Developing solvents: 1(a) ethanol–chloroform (2:98); 1(b) ethanol–chloroform (4:96); 2(a) n-butanol–glacial acetic acid–water (10:1:3) (Giacopello, 1965); 2(b) t-amyl alcohol–isoamyl alcohol–formic acid–water (1:1:1:5) (Raffauf, personal communication).

From the Department of Pharmacy, University of Strathclyde, Glasgow, C.1.

ISOLATION OF ALKALOIDS

The powdered stem (or root) bark (25 g) was extracted in a Soxhlet apparatus with light petroleum (bp 40–60°), then chloroform and, finally, ethanol (95%). The residue left on evaporation of the ethanol was extracted with hydrochloric acid (5%, 3×10 ml) and the acid solution extracted with chloroform (Gilbert & others, 1965) in a liquid-liquid extractor until extraction of alkaloids ceased. The aqueous phase was

TABLE 1. DISTRIBUTION OF TERTIARY AND QUATERNARY ALKALOIDS IN THE GENUS *Fagara*

Species	Alkaloids		Reference
	Tertiary	Quaternary	
South American			
**F. coco* Engl.	+	+	Comin & Deulofeu, 1954
**F. hyemalis* Engl.	−	+	Albonico, Kuck & Deulofeu, 1964
**F. naranjillo* Engl.	−	+	*Ibid.*
**F. nigrescens* Fries	−	+	*Ibid.*, Kuck, 1966
**F. pterota* Engl.	−	+	Albonico, Kuck & Deulofeu, 1964
**F. rhoifolia* Engl.	−	+	*Ibid.*
" "	+	+	Calderwood & Fish, 1966
F. tingoassuiba Hoehne	+	+	Riggs, Antonaccio & Marion, 1961
Australian			
F. brachyacantha Engl. (*Z. brachyacanthum* F. Muell.)	+	+	Jowett & Pyman, 1913
F. venenefica Engl. (*Z. veneneficum* F. M. Bailey)	+	+	Cannon, Hughes, Ritchie & Taylor, 1953
Asiatic			
F. ailanthoides Engl. (*Z. ailanthoides* Sieb. & Zucc.)	+	+	Tomita & Ishii, 1958
F. avicennae Lam. (*Z. avicennae* (DC.)	−	+	Arthur, Hui & Ng, 1959
F. hamiltoniana Engl. (*Z. hamiltonianum* Wall.)	−	+	Gopinath, Kohli, Khan & Kidwai, 1963
F. nitida Roxb. (*Z. nitidum* DC.)	+	+	Arthur, Hui & Ng, 1958
F. oxyphylla R. & C. (*Z. oxyphyllum*)	+	−	Chatterjee & Mukherjee, 1964
F. rhetsa Roxb. (*Z. rhetsa* DC.)	+	+	Chatterjee, Bose & Ghosh, 1959
F. schinifolia Engl. (*Z. schinifolium* Sieb. & Zucc.)	+	−	Ishii, 1961
F. semiarticulata St. John & Hosaka	+	+	Scheuer, Chang & Swanholm, 1962
African			
F. angolensis Engl.	+	−	Palmer & Paris, 1955
F. heitzii A. & P.	+	−	Palmer, 1956
F. leprieurii Engl.	+	−	*Ibid.*
F. macrophylla Engl.	+	−	*Ibid.*
F. melanacantha Engl.	+	+	*Ibid.*
F. parvifolia A. Cheval.	+	−	*Ibid.*
F. pubescens A. Cheval.	+	−	*Ibid.*
F. viridis A. Cheval.	+	−	Paris & Moyse-Mignon, 1948
F. xanthoxyloides Lam.	+	−	Paris & Moyse-Mignon, 1947

NOTE: Since this paper was submitted additional information on quaternary alkaloids of several South American *Fagara* species*, including *F. chiloperone* var. *angustifolia* Engl., has been published by Kuck, A. M. & Albonico, S. M. (1966). *Chemy Ind.*, 945–946.

basified (pH 8·0) with ammonia and re-extracted with chloroform until any residual tertiary alkaloids were removed. Finally the alkaline aqueous phase, still strongly alkaloid-positive, was extracted with n-butanol in a liquid-liquid extractor until free from alkaloids (Raffauf, personal communication).

TABLE 2. TERTIARY ALKALOIDS OF FOUR AFRICAN *Fagara* SPECIES

Species	Alkaloid	Reference
F. leprieurii Engl.	†Angoline †Angolinine Skimmianine	Palmer (1956)
F. macrophylla Engl.	Fagaramide †Fagaridine Skimmianine *†Un-named alkaloid *†Xanthofagarine	Goodson (1921) Paris & Moyse-Mignon (1951) Palmer (1956) King, Housley & King (1954) Paris & Moyse-Mignon (1951)
F. viridis A. Cheval.	Skimmianine	Paris & Moyse-Mignon (1948)
F. xanthoxyloides Lam.	†Artarine Fagaramide n-Isobutyldecenamide Skimmianine	Giacosa & Monari (1887) Thoms & Thümen (1911) Bowden & Ross (1963) Paris & Moyse-Mignon (1947)

† Alkaloids of unknown structure.
* Probably identical.

CHROMATOGRAPHY

The dried residues from each solvent extract were separately dissolved in chloroform (3 ml) or, in the case of the butanol extract, in ethanol (3 ml) and the solutions chromatographed, two-dimensionally, on alumina using the solvent systems 1(a) and 1(b) at 25°. The development distance was 10 cm for each solvent.

The butanol extracts were also chromatographed, two-dimensionally, on micro-granular cellulose using the developing solvents 2(a) and 2(b). Before each development the plates were equilibrated for 1 hr in the presence of the appropriate solvent. Development for a distance of 15 cm in each direction required approximately 120 and 140 min, respectively, at a constant temperature of 25°.

The following alkaloids were used as markers on both types of chromatoplates: α-allocryptopine, angoline, angolinine, 1-hydroxy-2,9,10-trimethoxy-*NN*-dimethylaporphinium chloride, α-(−)-*N*-methylcanadine chloride, (+)-tembetarine chloride and skimmianine.

Plates were examined in daylight and at 366 mμ; they were then sprayed with modified Dragendorff's reagent. The cellulose plates were sprayed first with ferric chloride reagent (1%, in ethanol 95%) to detect phenolic alkaloids (greyish-green to green areas) then with Dragendorff's reagent.

TABLE 3. IDENTIFICATION OF ALKALOIDS ON TWO-DIMENSIONAL ALUMINA CHROMATOGRAMS

Compound	Average Rf values		Colour	
	Solvent 1(a)	Solvent (1)b	In daylight	In ultraviolet light
All quaternary alkaloids ..	0·00	0·00		
Tertiary alkaloids				
A	0·06	0·13	red	dark purple
Angolinine	0·05	0·46		greenish-yellow
B	0·16	0·55		blue
C	0·53	0·55		
D	0·76	0·79		
Skimmianine	0·83	0·87		violet
Angoline	0·95	0·82	yellow	intense yellow
E*	0·88	0·97		pale blue

* Probably fagaramide.

Relative amounts of alkaloids were estimated by the size and colour density of the alkaloid areas after final spraying; results are given in Tables 3, 4 and 5.

TABLE 4. RELATIVE AMOUNTS OF TERTIARY ALKALOIDS IN BARKS OF *Fagara* SPECIES

	F. chalybea		*F. leprieurii*		*F. macrophylla*		*F. viridis*		*F. xanthoxyloides*	
Compound	stem	root	stem	root	stem	root	stem	root	stem	root
A			+	++	+	+	++	++		+
Angolinine ..	+	++++ ++	++++	++++ ++	+	++	+	+		+++
B		+	+		+	+++		+		+
C		+								++
D		+								
Skimmianine ..	+++	++++ ++	+	++++ ++	+	++++	++++ ++	++++ +	+++	++
Angoline ..	++++	++++ ++++	++++	++++ ++++	++	++++	++++	++++	++	+++
E*		+(?)				++				++

* Probably fagaramide.

Results and discussion

The extraction procedure removed both tertiary and quaternary alkaloids from all five *Fagara* species. Most of the tertiary bases were extracted by light petroleum and chloroform; the former extracted all base E (probably fagaramide) and partially extracted angoline, angolinine and skimmianine, while chloroform extracted most of the remainder of these latter alkaloids, together with bases designated A, B, C and D. The ethanol extract contained mainly quaternary compounds with the small amount of remaining tertiary bases. These tertiary bases, together with traces of quaternary alkaloids, were removed from the dried ethanol extract by extraction with chloroform, firstly from an acidic and later from an alkaline aqueous phase. The first extraction removed most of the alkaloids and gave a much cleaner chloroform solution than was subsequently obtained in the alkaline extraction. A final extraction of the alkaline phase with n-butanol yielded a solution containing only the quaternary alkaloids.

Chromatography on alumina distinguished between tertiary and quaternary alkaloids; under the conditions used, the latter remained at the point of application while all tertiary bases moved and excellent separation of these was obtained on two-dimensional chromatograms. With cellulose powders containing binding agents no separation of quaternary alkaloids was obtained; only continuous streaks of alkaloid were observed. Micro-granular cellulose plates, prepared without a binding agent (Giacopello, 1965), gave excellent separation of these compounds.

Fagara leprieurii, *F. macrophylla*, *F. viridis* and *F. xanthoxyloides* all previously known to contain tertiary bases, were shown to contain significant amounts of quaternary alkaloids; *F. chalybea*, not previously

TABLE 5. RELATIVE AMOUNTS OF QUATERNARY ALKALOIDS IN BARKS OF *Fagara* SPECIES

	Average Rf values†	*F. chalybea*		*F. leprieurii*		*F. macrophylla*		*F. viridis*		*F. xanthoxyloides*	
Compound	Solvent 2(a) 2(b)	stem	root	stem	root	stem	root	stem	root	stem	root
Q1	0·27, 0·12			+	+	+	+	+	+		
Q2*	0·39, 0·21			+++	+++	+	+	+++	+++	++	+
Q3*	0·38, 0·28	+	+	+++	+++		+++	+++	++++	++++	++
Q4*	0·46, 0·30		+	++		+				+++	++
Q5*	0·46, 0·36	+++	++	+++	++	++		+	+++		
Q6	0·36, 0·35										+++
Q7	0·48, 0·37	++++	++++	++++	+++	++++	++++	+++	+++	+++	++++
Q8	0·45, 0·47							++++	++++		
Q9	0·52, 0·50					+	++				
Q10	0·56, 0·53	+	++	++	++	++++	++++	++	+++	++	++
Q11	0·59, 0·68									+	++
Q12	0·71, 0·74	+	+	+++	++	+	++	+	+	++	+

Q2 Probably 1-hydroxy-2,9,10-trimethoxy-*NN*-dimethylaporphinium chloride.
Q4 Probably (+)-tembetarine chloride.
* Phenolic alkaloids.
† Obtained from two-dimensional cellulose chromatograms using solvents 2(a) and 2 (b).

investigated, was also shown to contain both types of compound. In most instances the root bark contained a larger total amount of alkaloid and a larger number of individual alkaloids than the stem bark of the same species (Tables 4 and 5).

TERTIARY ALKALOIDS

On thin-layer chromatoplates, all five species showed the presence of three principal tertiary bases corresponding to angoline, angolinine and skimmianine, though the amount of angolinine in *F. viridis* was small. The furoquinoline base skimmianine is the most common alkaloid not only in the genus *Fagara* but also in the family Rutaceae. Appreciable amounts of alkaloid A were found in all species except *F. chalybea*. The remaining tertiary bases, B, C, D and E were of minor importance and their distribution varied amongst the five species. The tertiary protopine base α-allocryptopine, reported in the two Australian species *F. brachyacantha* and *F. venenefica* and in the two South American species *F. coco* and *F. rhoifolia* (Table 1), appeared to be absent from these African species.

QUATERNARY ALKALOIDS

Quaternary alkaloids (unidentified) have previously been reported in but one African *Fagara* species, *F. melanacantha* (Palmer, 1956). We have now found such compounds as a major group of alkaloids in both the stem and root barks of the species investigated. Some of the quaternary alkaloids are phenolic and one of these appeared to be identical with 1-hydroxy-2,9,10-trimethoxy-*NN*-dimethylaporphinium chloride. When an authentic sample was admixed with the butanol extracts, from all species except *F. chalybea* there resulted an area of increased intensity for the spot Q2 (Table 5). This compound has been reported previously in the two South American species *F. tingoassuiba* (Riggs, Antonaccio & Marion, 1961) and *F. rhoifolia* (Calderwood & Fish, 1966). The compound Q4, found in *F. chalybea*, *F. leprieurii* and *F. xanthoxyloides*, appears to be identical with the quaternary benzylisoquinoline alkaloid (+)-tembetarine chloride, previously isolated from the South American species *F. naranjillo*, *F. hyemalis*, *F. nigrescens*, *F. pterota* and *F. rhoifolia* (Albonico, Kuck & Deulofeu, 1964). The quaternary protoberberine base α-(−)-*N*-methylcanadine chloride found in *F. rhoifolia* (Calderwood & Fish, 1966), *F. brachyacantha* (Jowett & Pyman, 1913) and in *F. venenefica* (Cannon, Hughes, Ritchie & Taylor, 1953) did not correspond, on two-dimensional chromatograms (Rf values 0·68, 0·71), with any of the quaternary alkaloids of the barks examined.

The five African *Fagara* species examined contain certain tertiary and quaternary alkaloids in common, but of the various related chemical groups of alkaloids known to be present in the genus *Fagara*, not all appear to be represented in these African species.

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