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# Alkaloids from two Nigerian species of *Fagara*

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Skimmianine, chelerythrine, nitidine, 1-hydroxy-2,3-dimethoxy-10-methylacridan-9-one and 1-hydroxy-3-methoxy-10-methylacridan-9-one have been isolated from the root and stem barks of *Fagara rubescens*. Using thin-layer chromatography and high voltage electrophoresis, a comparison of the chloroform-insoluble quaternary alkaloids of this species has been made with those from *F. leprieurii*. The distribution of the alkaloids within the barks and the chemotaxonomic significance of the alkaloids in the two species are discussed.

*Fagara rubescens* (Planch. ex Hook. f.) Engl. (syn. *Zanthoxylum rubescens* Planch. ex Hook. f.) is a small tree indigenous to Western Tropical Africa where the bark has been used as a toothache remedy: bark from *F. leprieurii* (Guill. et Perr.) Engl. (syn. *Zanthoxylum leprieurii* Guill. et Perr.) has been used similarly and for the treatment of coughs and colds (Oliver, 1960).

## MATERIALS

The root and stem barks of *Fagara rubescens* and *F. leprieurii*, collected in Nigeria for the Tropical Products Institute, London, were authenticated at source.

The barks, from which all adhering epiphytes had been removed, were separately ground to coarse powders. In the case of the root bark of *Fagara rubescens* a quantity of loosely-adhering cork was removed and ground separately.

*Thin-layer chromatography and high voltage electrophoresis.* These were performed using the methods previously described (Calderwood & Fish, 1969), in the latter technique using a barbitone buffer of pH 7·4.

*Identification of isolated compounds.* Alkaloids were identified by comparative t.l.c. (3 systems), mixed melting points and comparison of ultraviolet and infrared spectra with those obtained for authentic samples (Calderwood, Finkelstein & Fish, 1970; Fish & Waterman, 1971a). Melting points (uncorrected) were determined on a Kofler hot stage, ultraviolet spectra (in ethanol) were recorded on a Unicam Stereoscan S.P. 800 and infrared spectra (Nujol mull) on a Perkin-Elmer 157B instrument.

## METHOD

*Isolation of the chloroform-soluble alkaloids of* Fagara rubescens. The powdered barks (root bark 1 kg, stem bark 1 kg and cork 100 g) were separately extracted to exhaustion (48 h) in a Soxhlet with light petroleum (b.p. 40–60°) and then with chloroform. Each extract was concentrated under reduced pressure to a volume of 250 ml.

For each concentrate an aliquot (100 ml) was extracted successively with 2N hydrochloric acid (3 × 50 ml, 2 × 25 ml), the final acid wash being allowed to stand

overnight in contact with the original extract. The combined acid extracts were filtered and the precipitated base hydrochlorides subjected to column chromatography on alumina (Woelm, activity II) (20 g/g of crude base precipitate packed in chloroform–methanol (98:2). Elution with the same solvent gave chelerythrine in the first 50 ml, well separated from nitidine, which was eluted in the subsequent 150 ml. Chelerythrine was crystallized as the chloride from 2N hydrochloric acid–ethanol to give yellow needles, melting point 202° (Lit m.p. 202–203°, Cannon, Hughes & others, 1953). Nitidine was crystallized as the nitrate from 6N nitric acid–ethanol to give yellowish green needles, melting point 276–278° (Lit m.p. 277–278°, Gopinath, Khan & others, 1963). Yields are indicated in Table 1.

The bulked acid fractions from each initial extract were separately made alkaline with strong solution of ammonia and re-extracted with chloroform. Evaporation of the chloroform extracts under reduced pressure followed by crystallization from methanol gave (stem bark and the inner root bark) the furoquinoline alkaloid skimmianine, melting point 176–177° (Lit m.p. 176°, Deulofeu, Labriola & de Lange, 1942).

A second aliquot (100 ml) of each initial concentrate was subjected directly to column chromatography on Silica Gel G–Celite 545 (1:1) (250 g for each 100 ml of concentrate) packed in benzene–ethyl acetate (9:1). Elution with the same solvent gave 1-hydroxy-2,3-dimethoxy-10-methylacridan-9-one which was crystallized from ethyl acetate to give fine, yellow needles, melting point 175–176° (Lit m.p. 175–176°, Pakrashi, Roy & others, 1961). Elution with benzene–ethyl acetate (1:1) gave a second alkaloid 1-hydroxy-3-methoxy-10-methylacridan-9-one, crystallized from ethyl acetate to give yellow clusters, melting point 175–176° (Lit m.p. 174–175°, Drummond & Lahey, 1949) giving no depression on admixture with an authentic specimen.

The concentrations of the various alkaloids obtained from the barks are given in Table 1 together with yields previously reported from *Fagara leprieurii* (Fish & Waterman, 1971a).

Table 1. *Percentages of chloroform–soluble alkaloids present in the root and stem barks of* Fagara rubescens *and* F. leprieurii *and in the separated cork from the root bark of* F. rubescens.

| | *F. rubescens* | | | *F. leprieurii* | |
|---|---|---|---|---|---|
| | Cork | Root, inner bark | Stem bark | Root bark | Stem bark |
| Skimmianine .. .. | absent | 0·029 | 0·015 | 0·003 | 0·001 |
| Chelerythrine .. .. | 0·031 | 0·029 | trace | 0·017 | trace |
| Nitidine .. .. .. | 0·006 | 0·009 | trace | 0·004 | trace |
| Acridone a* .. .. .. | absent | 0·094 | 0·081 | 0·031 | 0·020 |
| Acridone b* .. .. | absent | 0·062 | 0·028 | 0·008 | 0·005 |

* a = 1-Hydroxy-2,3-dimethoxy-10-methylacridan-9-one.
b = 1-Hydroxy-3-methoxy-10-methylacridan-9-one.

*Examination of quaternary alkaloids.* The barks of *Fagara rubescens* and *F. leprieurii*, previously extracted with light petroleum and chloroform, were further extracted with methanol, the extracts concentrated under reduced pressure and

subjected to a partial purification process in which the concentrates were mixed with Amberlite IRC-50 ion exchange resin (75% $Na^+$ form) from which they were eluted with methanol containing hydrochloric acid (5%) (Albonico, Kuck & Deulofeu, 1964).

The concentrated extracts were then subjected to thin-layer chromatography (cellulose, 3 systems) and high voltage electrophoresis. The results obtained are given in Table 2.

Table 2. *Thin layer and electrophoretic separation of methanol-soluble alkaloids from* Fagara *barks.*

| Reference compounds | Alkaloid movement | *F. rubescens* Root bark | *F. rubescens* Stem bark | *F. leprieurii* Root bark | *F. leprieurii* Stem bark |
|---|---|---|---|---|---|
| Magnoflorine .. .. | a 0·24<br>b 0·30<br>c 0·24<br>d 0·30 | a 0·25<br>b 0·31<br>c 0·24<br>d 0·30 | a 0·25<br>b 0·31<br>c 0·24<br>d 0·35 | a 0·22<br>b 0·30<br>c 0·25<br>d 0·35 | a 0·25<br>b 0·31<br>c 0·25<br>d 0·40 |
| Alkaloid A .. .. .. | | a, b, c ?<br>d 7·70 | a, b, c ?<br>d 7·80 | a, b, c −*ve*<br>d | a, b, c −*ve*<br>d |
| *Tembeterine .. .. | a 0·81<br>b 0·55<br>c 0·57<br>d 8·50 | a 0·83<br>b 0·57<br>c 0·58<br>d 8·60 | a 0·84<br>b 0·56<br>c 0·59<br>d 8·65 | a 0·83<br>b 0·56<br>c 0·59<br>d 8·50 | a 0·88<br>b 0·56<br>c 0·58<br>d 8·60 |
| Candicine .. .. .. | a 0·90<br>b 0·45<br>c 0·47<br>d 14·70 | a 0·91<br>b 0·45<br>c 0·47<br>d 14·50 | a 0·90<br>b 0·45<br>c 0·47<br>d 14·60 | a, b, c −*ve*<br>d | a, b, c −*ve*<br>d |

(i) a = $R_F$ in 0·1N HCl.
(ii) b = $R_F$ in butanol–pyridine–water (6 : 4 : 3).
(iii) c = $R_F$ in butanol saturated with 2N HCl.
(iv) d = distance travelled in high voltage electrophoresis (cm).
Alkaloid A may be either *N*-methylcorydine or *N*-methylisocorydine for both of which d = 7·75.
* For previous work on *F. leprieurii*, Calderwood & Fish, 1966.

## DISCUSSION

The five chloroform-soluble alkaloids isolated from the bark of *Fagara rubescens* are identical with those previously reported from the bark of *F. leprieurii* (Fish & Waterman, 1971a). *F. rubescens* is thus the second African species of this genus shown to contain the acridone type of alkaloid, a type previously not reported from any other *Fagara* species.

The distribution of the alkaloids, indicating the presence of greater concentrations in the root bark than in the stem bark, confirms the results previously obtained for other members of this group (Calderwood & Fish, 1966; Fish & Waterman, 1971b). The presence of large quantities of chelerythrine, together with nitidine, in the cork of the root bark and the absence of all the other bases (including the methanol-soluble quaternary alkaloids) is noteworthy. These same two alkaloids are also present in the remainder of the root bark but almost absent from the stem bark although this contains appreciable quantities of the other alkaloids.

Information on alkaloid patterns in the genus *Fagara* may have chemotaxonomic significance since the present classification on African species, totalling about 40, is

considerably confused. The two species presently considered are morphologically very similar (cf. Oliver, 1868; Hutchinson & Dalziel, 1954). Chemical similarities obviously exist in that both species contain the dopamine-derived chelerythrine and nitidine (probably also tembetarine and magnoflorine) as well as the anthranilic acid-derived acridone alkaloids. To date these last have not been demonstrated in any other *Fagara* species. Differences are also shown by the preliminary work (Table 2) which suggests that the tyramine-derived candicine is present only in *F. rubescens*.

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