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ALKALOIDS FROM ARGENTINE *FAGARA* SPECIES[1]

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Abstract—The alkaloids of seven of the eight species of *Fagara* growing in Argentina have been investigated. All the bases isolated and characterized were quaternary and belonged either to the simple phenylethylamines or to the benzylisoquinolines. In the last group one benzylisoquinoline, several aporphines, two berberines and two benzophenanthridine alkaloids were identified (Table 1). Two of them, tembetarine and *N*-methylcorydine were found for the first time in plants. Some considerations are made on the distribution of these alkaloids among *Fagara* and *Zanthoxylum* species.

INTRODUCTION

EIGHT species of *Fagara* (Rutaceae) grow in Argentina.[2] All of them belong to the Macqueria section, three are included in the Pterota subsection, and the other five in the Paniculatae (Table 1).

Only one, *F. coco* (Gill.) Engl., has been previously investigated[3] and two types of tertiary alkaloids were isolated from leaves and young twigs: the furoquinolines γ-fagarine and skimmianine,[4] and allocryptopine (α-fagarine) and fagarine II of the protopine group.[5] The main alkaloid in the bark was the quaternary aporphine base, *N*-methylisocorydine[6] with small amounts of skimmianine and allocryptopine.[7] Other bases of unknown structure were described by Stuckert[3] and Redeman.[5a]

We now report an investigation of the bases present in the bark of six more species: *F. chiloperone* var. *angustifolia* (Engl.) Engl., *F. hyemalis* (St. Hil.) Engl., *F. naranjillo* (Griseb.) Engl., *F. nigrescens* Fries, *F. pterota* L. and *F. rhoifolia* (Lam.) Engl. The bark of *F. coco* was also reinvestigated and the new results obtained are included here. We were unable to obtain material from the remaining species *F. riedeliana* (Engl.) Engl.

With the exception of *F. coco*, whose leaves are rich in alkaloids,[3] acid extracts from the leaves of all other species investigated gave only a very faint reaction for alkaloids, and we were unable to characterize individual bases.

[1] A previous communication has been published in *Chem. Ind. (London)* 945 (1966).

[2] (a) In this paper the classification of the *Fagara* genus by A. ENGLER *Die naturlichen Pflanzenfamilien* (Edited by A. ENGLER and K. PRANDL), Vol. 3, Part 4/5, p. 95. Engelmann, Leipzig (1897), has been followed, supplemented by the *Index Kewensis*. (b) The Argentine *Fagara* species have been discussed in detail by M.G. ESCALANTE, *Bol. Soc. Arg. Botán.* **9**, 291 (1961).

[3] G. V. STUCKERT, *Invest. Lab. Quim. Biol.* **1**, 69 (1933). Facultad de Ciencias Médicas, Universidad Nacional de Córdoba.

[4] V. DEULOFEU, R. LABRIOLA and J. DE LANGHE, *J. Am. Chem. Soc.* **64**, 2326 (1942).

[5] (a) C. E. REDEMAN, B. B. WISEGARVER and G. A. ALLES, *J. Am. Chem. Soc.* **71**, 1030 (1949). (b) J. COMIN and V. DEULOFEU, *Tetrahedron* **6**, 63 (1959); D. GIACOPELLO, V. DEULOFEU and J. COMIN, *Tetrahedron* **20**, 2971 (1964).

[6] J. COMIN and V. DEULOFEU, *J. Org. Chem.* **19**, 1774 (1954).

[7] E. M. BARILARI and J. COMIN, *Anales Asoc. Quim. Arg.* **43**, 180 (1955).

TABLE 1. ALKALOIDS ISOLATED FROM ARGENTINE *Fagara* SPECIES*

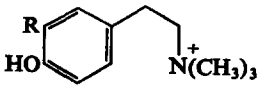
Species and location†	Candicine (I)	Corynoine (II)	Tembetarine (III)	Magnoflorine (IV)	*N*-methylisocorydine (V)	*N*-methylcorydine (VI)	Laurifoline (VII)	Xanthoplanine (VIII)	Palmatine (IX)	Berberine (X)	Chelerytrine (XI)	Nitidine (XII)	A-82
(I) Subsect. Pterota (P. Brown) Engl.													
(1) *F. hyemalis* (St. Hil.) Engl.													
(a) Corrientes†, May 1963	0·60	0·10	3·60	0·22	—	—	0·07	—	—	—	0·004	—	+§
(b) Misiones, August 1964	0·49	0·16	2·10	0·62	—	—	0·11	0·17	—	—	0·002	—	+
(2) *F. nigrescens* Fries													
(a) Salta, January 1963	0·10	—	1·50	—	0·005	0·03	—	0·26	—	—	—	0·004	+
(3) *F. pterota* L.													
(a) Chaco, February 1962	0·70	—	0·90	0·11	0·14	—	1·50	—	—	—	0·013	0·003	+
(II) Subsect. Paniculatae Engl.													
(4) *F. chiloperone* var. *angustifolia* (Engl.) Engl.													
(a) Misiones, August 1964	0·40	—	1·20	0·04	—	—	0·07	—	—	—	0·033	0·005	+
(5) *F. coco* (Gill.) Engl.‡													
(a) Cordoba, February 1962	0·02	—	—	0·32	1·20	—	—	—	0·030	0·002	0·004	0·010	—
(b) Cordoba, February 1964	0·02	—	—	0·35	1·10	—	—	—	0·030	0·002	0·010	0·009	—
(6) *F. naranjillo* (Griseb.) Engl.													
(a) Chaco, October 1961	—	—	2·00	—	—	—	—	—	—	—	0·005	—	—
(b) Chaco, February 1962	—	—	1·80	—	—	—	—	—	—	—	0·005	—	—
F. naranjillo var. *paraguariensis* (Chodat et Hassler) Escal.													
(c) Corrientes, January 1964	—	—	1·80	0·11	—	—	—	—	—	—	0·004	—	—

(7) *F. rhoifolia* (Lam.) Engl.													
(a) Chaco, February 1962	0·94	—	0·05	0·07	0·05	—	—	—	—	—	—	—	+
(b) Buenos Aires, August 1963	0·20	—	—	0·08	—	—	—	—	—	—	—	—	—
(c) Buenos Aires, November 1965	0·15	—	—	0·25	0·03	—	—	—	—	—	—	—	—
F. rhoifolia var. *petiolulatum* (Engl.) Engl.													
(d) Misiones, August 1961	0·05	—	—	0·35	0·04	—	—	—	—	—	0·020	0·007	+
(e) Misiones, August 1961	0·05	—	—	0·31	0·03	—	—	—	—	—	0·020	0·007	+
(f) Corrientes, January 1964	0·19	—	—	0·10	—	—	—	—	—	—	0·006	0·003	—
(g) Misiones, August 1964	0·10	—	—	0·20	—	—	—	—	—	—	0·004	0·003	—
(h) Misiones, January 1965	0·10	—	—	0·35	0·03	—	—	—	—	—	0·004	0·007	—
(i) Misiones, January 1965	0·15	—	—	0·30	0·03	—	—	—	—	—	0·006	0·003	—

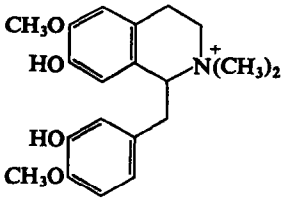
* Yields are expressed in % of dry plant material and refer to the weight of salts isolated after the first crystallization as described in the experimental part.
† Geographical names refer to the Argentine Provinces where the material was collected.
‡ The presence of the tertiary bases skimmianine and allocryptopine (α-fagarine) was confirmed and fagarine II was also found to be present in the bark.
§ (+) A qualitative indication of presence.

RESULTS AND DISCUSSION

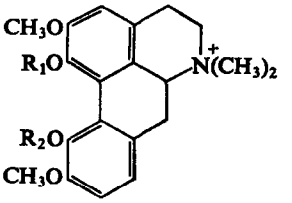
The bases isolated and identified are mentioned in Table 1, and the structures of their cations are indicated (I–XII). The presence, in several species, of a group of bases with a high water solubility which were almost insoluble in solvents of low polarity is indicated in the last column under A-82. They were found difficult to purify by the methods employed, and reserved for further study. No other bases were detected. If the furoquinolines alkaloids found in *Fagara coco* and the simple phenylethylamines (protoalkaloids) candicine (I) and coryneine (II) are excepted, all other bases belong to the benzylisoquinoline group using this term in a broad sense.



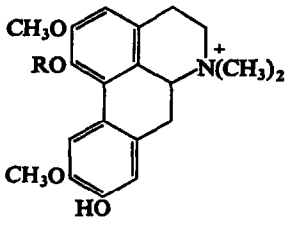
Candicine (I): R=H
Coryneine (II): R=OH



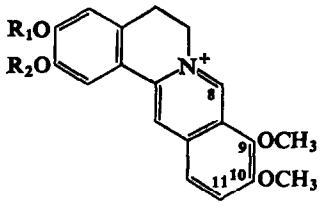
Tembetarine (III)



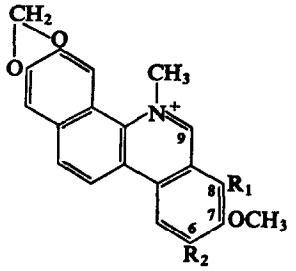
Magnoflorine (IV): $R_1=R_2=H$
N-Methylisocorydine (V): $R_1=CH_3$, $R_2=H$
N-Methylcorydine (VI): $R_1=H$, $R_2=CH_3$



Laurifoline (VII): R=H
Xanthoplanine (VIII): $R=CH_3$



Palmatine (IX): $R_1=R_2=CH_3$
Berberine (X): $R_1+R_2=CH_2$



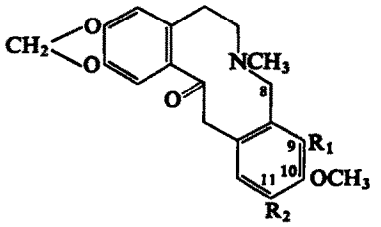
Chelerytrine (XI): $R_1=OCH_3$, $R_2=H$
Nitidine (XII): $R_1=H$, $R_2=OCH_3$

As a result of this work, six bases have been isolated for the first time from *Fagara* spp.: the two phenylethylamines mentioned above, (+)-tembetarine (III), (+)-*N*-methylcorydine (VI), (+)-xanthoplanine (VIII) and palmatine (IX). Two of them, tembetarine (III) and *N*-methylcorydine (VI), are new natural bases. The former was found in all species studied, with the exception of *F. coco*. It has recently been isolated also from *Phoebe porphyria* (Gris.) Mez. (Lauraceae).[8] The determination of its structure as (+)-*N*-methylreticuline and its oxidation to the aporphine alkaloid (+)-laurifoline (VII) has already been reported.[9] *N*-methylcorydine (VI) was isolated only, in a very small amount, from *F. nigrescens*.[10]

In view of the strong botanical relationship between the genus *Fagara* and *Zanthoxylum*, it is interesting to note that only three of the twelve alkaloids included in Table 1, magnoflorine (IV), xanthoplanine (VIII) and berberine (X), have been also isolated from *Zanthoxylum* spp.

[8] A. M. KUCK, Unpublished results.
[9] S. M. ALBÓNICO, A. M. KUCK and V. DEULOFEU, *Chem. Ind. (London)* 1580 (1964); *Ann. Chem.* **685**, 200 (1965).
[10] A. M. KUCK, *Chem. Ind. (London)* 118 (1966).

Some comments on the type and distribution of the bases isolated are pertinent. One common feature is that if we exclude the furoquinoline and protopine alkaloids from *F. coco*, all other bases present in the *Fagara* species worked are quaternary and, with the exception of berberine (X) and palmatine (IX), *N*-methylated. Enzyme systems responsible for quaternization through *N*-methylation seem to be rather active in those species. If the corresponding tertiary or secondary bases are present, their amount must be below the limit of identification given by the chromatographic methods employed. Even the tertiary bases allocryptopine (XIII) and fagarine II (XIV), present in *F. coco*,[5] have passed through the process of a second *N*-methylation, as the first *N*-methyl group should have been used in a precursor, to form the carbon atom 8 of their ten member heterocyclic ring.[11]



Allocryptopine (XIII): $R_1=OCH_3$, $R_2=H$
Fagarine II (XIV): $R_1=H$, $R_2=OCH_3$

It is of interest that so many quaternary alkaloids are present in the species investigated, specially because they are usually considered not to be very active metabolically. On the other hand, it is well known that dopamine, the primary base which corresponds to coryneine, and reticuline, the tertiary benzylisoquinoline alkaloid related to tembetarine, are common precursors of several alkaloids.

Some comments can also be made on the distribution of the different groups of alkaloids and of individual bases. In the group of protoalkaloids the wide distribution of the monophenolic base candicine (I), found in all species, with the exception of *F. naranjillo*, contrasts with the presence of its hydroxylated derivative coryneine (II), in only one species, *F. hyemale*. One can speculate that the original primary amine, dopamine, is used much faster than tyramine in the biogenesis of more elaborated bases, with the result that only a small amount is quaternized by *N*-methylation into coryneine (II).

Although the number of species investigated is too small to make a generalization, it is also an interesting point that the substitution pattern of the different aporphine alkaloids isolated shows a co-relation with the assignment of the species to the subsections, Pterota or Paniculatae, of the *Fagara* genus.

It can be seen from Table 1 that while the bases with the corytuberine substitution pattern [magnoflorine (IV), *N*-methylisocorydine (V) and *N*-methylcorydine (VI)] are found in both subsections, magnoflorine (IV) being quantitatively predominant, the alkaloids with a substitution of the glaucine type [laurifoline (VII) and its mono-*O*-methyl ether, xanthoplanine (VIII)] are concentrated in the Pterota subsection, the only exception being laurifoline (VII), which has also been found in *F. chiloperone* which belong to the Paniculatae. It is

[11] A. R. BATTERSBY, *Proc. Chem. Soc.* 189 (1963); D. H. R. BARTON, *ibid.* 293 (1963); D. H. R. BARTON, R. H. HESSE and G. W. KIRBY, *ibid.* 267 (1963) and *J. Chem. Soc.* 6379 (1965); A. R. BATTERSBY, R. J. BATTERSBY, R. J. FRANCIS, M. HIRST and J. STAUNTON, *Proc. Chem. Soc.* 268 (1963).

interesting that the other mono-*O*-methyl ether of laurifoline, was first isolated from *F. tinguassoiba*,[12] which belong to the Pterota subsection as well.

Calderwood and Fish[13] have recently reported the isolation of the quaternary aporphine base present in *F. tinguassoiba* from an extract of the bark of *F. rhoifolia* together with small amounts of α-allocryptopine and of the quaternary alkaloid (−)-*N*-methylcanadine. However, in our extracts from the latter species these bases could not be detected even by paper chromatography.

Although it is difficult to explain these differences, it is important to remember that *F. rhoifolia* is the most variable of the species investigated, and a great number of synonyms, varieties and forms have been described. We have examined nine specimens with the results indicated in Table 1. Six of them (*d-i*) belong to *F. rhoifolia* var. *petiolulatum* (Engl.) Engl.[14] They were collected near the Parana River, in the North eastern sub-tropical provinces of Corrientes and Misiones and are characterized by their elliptic, narrow and dull folioles, with crenate margins. These specimens contained the benzophenanthridine alkaloids, chelerythrine (XI) and nitidine (XII) which were absent from the others (*a-c*). Specimen *a*, collected in the neighbouring region (near the Parana River, Province of Chaco), has lanceolate, multicrenate and bright folioles, which turn reddish when dry. These characteristics coincide with the original description of *F. niederleinii* Engl.,[15] which is now considered to be synonymous with *F. rhoifolia*. It was the only specimen containing tembetarine (III) and A-82 bases. Specimens *b* and *c*, collected 100 Km north of the city of Buenos Aires, are representative of a particular variety, which is peculiar to that region and features oblong folioles. Their content of alkaloids was rather poor, and tembetarine (III) was not present.

It is evident that *F. coco* has a unique position among the Argentine species of *Fagara*, because it contains furoquinoline, protopine and berberine bases, three types of alkaloids absent in the other species. *F. coco* is also differentiated by its morphology and ecology. It can grow in a dry warm climate, with scarce summer rains, in a xerophitic deciduous forest, while the remaining species are found only in a subtropical rain forest. It is the only species with large and diffuse inflorescences and with leaves containing many translucent glandular points distributed in the whole foliar limb.[16]

The presence in *F. coco* of the bases, chelerythrine (XI) and nitidine (XII), together with berberine (X) and palmatine (IX) is interesting in view of the recent progress made on the biogenesis of the phenanthridine alkaloids.[17] In changing from a berberine to a benzophenathridine structure, carbon atom 8 of the berberine skeleton gives rise to carbon atom 9 of the benzophenanthridines. While chelerythrine (XI) has a substitution pattern related to berberine (X), palmatine (IX) and allocryptopine (XIII)—all of them found in *F. coco*—the hypothetical precursor of nitidine (XII), which is related to fagarine II (XIV), also found in that plant,[5] has never been detected.

The presence of chelerythrine (XI) in all species of *Fagara* investigated, while berberine (X) and palmatine (IX) are only found in *F. coco*, could indicate different metabolic rates occur within the pool of berberine and benzophenathridine alkaloids.

[12] N. V. RIGGS, L. ANTONACCIO and L. MARION, *Can. J. Chem.* **39**, 1330 (1961).
[13] J. M. CALDERWOOD and F. FISH, *Chem. Ind. (London)* 237 (1966).
[14] See (2a) p. 117, based on *Zanthoxylum rhoifolium* Lam. var. *petiolulatum* A. ENGLER, In *Martius, Flora Brasiliensis* **12**, 162 (1874).
[15] A. ENGLER, *Bot. Jahrbücher* **21** (5), 24 (1896).
[16] J. GILLIES, *Botan. Miscellany* **3**, 168 (1833).
[17] R. B. TURNER and R. B. WOODWARD, *The Alkaloids* (Edited by R. H. F. MANSKE and H. L. HOLMES), Vol. 3, p. 57. Academic Press, New York (1958); A. R. BATTERSBY, R. J. FRANCIS, E. A. RÚVEDA and J. STAUNTON, *Chem. Commun.* 89 (1965).

EXPERIMENTAL

Methods

The plant material was air dried and kept at room temperature.

The following chromatographic systems were employed on Whatman paper No. 1. (I) 0·1 N HCl, for ascending chromatography; (II) *n*-butanol saturated with 2 N HCl, and (III) *n*-butanol:pyridine:water (6:4:3), for descending chromatography. The chromatograms were observed with u.v. light (360–370 nm) before and after exposure to ammonia vapor. The bases were detected with iodoplatinic acid reagent,[18] Dragendorff reagent,[19] and diazotized sulfanilic acid. Table 2 gives the R_f values found for the different alkaloids. Solvent (I) was also used for column chromatography on cellulose. All evaporations were carried out under diminished pressure at the lowest possible temperature.

TABLE 2. CHROMATOGRAPHIC CHARACTERISTICS OF THE QUATERNARY ALKALOIDS FROM *Fagara* SPECIES

Alkaloids	R_f values*			Colors with reagents†, §		Fluorescence under u.v. light‡, §	
	(I)	(II)	(III)	Iodoplatinic acid	Diazotised sulfanilic acid	Direct	After NH_3
Candicine	0·86	0·54	—¶	Grey	Red	—	—
Coryneine	0·86	0·34	0·35	Grey	Pale-brown	—	—
Tembetarine	0·75	0·61	—¶	Green	Orange	—	—
Magnoflorine	0·20	0·34	0·13	Brown	Yellow	—	Blue
N-methylisocorydine	0·62	0·58	0·51‖	Brown	Red	—	Light-blue
N-methylcorydine	0·67	0·68	0·59	Brown	—	—	Light-blue
Laurifoline	0·07	0·21	0·45	Bluish-brown	Pale-brown	—	Dark-blue
Xanthoplanine	0·21	0·46	0·58	Bluish-brown	Pale-brown	—	Dark-blue
Palmatine	0·12	0·22	0·54	Light-brown	—	Yellow	Yellow
Berberine	0·13	0·33	0·54	Light-brown	—	Yellow	Yellow
Chelerythrine	0·10	0·36	0·91	Light-brown	—	Orange	Pale-orange
Nitidine	0·05	0·05	0·42‖	Light-brown	—	Yellow	Pale-yellow

* Whatman paper No. 1. Systems, I: 0·1 N HCl (ascending), II: butanol saturated with 2 N HCl (descending), III: butanol: pyridine: water 6:4:3 (descending).
† All spots gave positive reaction with Dragendorff reagent according to Munier and Macheboeuf.
‡ 360–370 nm.
§ (—) no color or fluorescence were observed.
‖ With tailing.
¶ Long spot, useless for characterization.

In many cases the bases were isolated as picrates and styphnates which were converted into chlorides and iodides, employing the method described by Bobbitt.[20]

The known alkaloids were identified by comparing the properties of crystalline derivatives with authentic samples except in a few cases. Mixed m.p., determination of the R_f with different solvent systems, u.v., i.r., and NMR spectra, were the criteria mainly used. As it was not possible to obtain samples of coryneine, laurifoline, and xanthoplanine, a larger number of derivatives of those bases was prepared. M.p. are uncorrected.

Extraction

The following is a general description of the process used for the extraction of the bases and the first stages of purification.

The bark, ground to 9 mesh, was well extracted with light petroleum (b.p. 60–70°) and the extracts reserved for further studies. It was then extracted several times with boiling methanol, until the alkaloidal reaction was negative or very faint.

[18] L. R. GOLDBAUM and L. KAZYAK, *Anal. Chem.* **28**, 1289 (1956).
[19] R. MUNIER and M. MACHEBOEUF, *Bull. Soc. Chim. Biol.* **38**, 846 (1951).
[20] J. M. BOBBITT, *J. Org. Chem.* **22**, 1729 (1957).

The combined methanol extracts were filtered and evaporated, and in all cases a heavy syrupy residue remained. The residue was treated with 0·1 N HCl, and by shaking for 30 min a fine suspension was obtained, which was filtered (Celite). The insoluble portion was re-extracted twice in the same way. The acid extract (A) contained all the alkaloids present. An aliquot of this extract was reserved for the investigation of benzophenanthridine alkaloids. To the remaining solution, 4 N NaOH was added to pH 6. A precipitate was produced which was separated by filtration, and the filtrate passed through a column of Amberlite IRC-50 (50% H^+, 50% Na^+, v/v), employing 1 l. of the resin per kg of bark, which retained all the bases. It was washed with water, with 50% ethanol and finally with 96% ethanol. The washings were negative to Dragendorff reagent.

The bases were eluted from the column with ethanolic 0·6 N HCl until a negative test for alkaloids was observed. The acid eluate was evaporated to dryness and the solid residue extracted with boiling ethanol, which dissolved the chlorides of the alkaloids, while most of the sodium chloride remained insoluble. The ethanolic solution (B) was again evaporated to dryness, collecting by filtration any alkaloid chlorides which crystallized during the operation. The bases present in the residue (C) obtained were examined by paper chromatography (solvents I and II). The residue (C) was then treated according to its complexity, and the bases separated and identified as described below.

Separation and Identification of the Phenylethylamines, and of the Benzylisoquinoline and Aporphine Alkaloids

Fagara rhoifolia

The residue (C) contained four bases: candicine, tembetarine, magnoflorine, and *N*-methylisocorydine. It was dissolved in 0·1 N HCl, and chromatographed on a column of cellulose (48 × 570 mm). Fractions containing individual bases appeared in the order given below; they were combined and further purified as indicated below.

Candicine. On evaporation, the corresponding fractions afforded an amorphous solid, which crystallized easily as chloride from ethanol. M.p. 284–285°, λ_{max}^{EtOH} 278 nm (log ϵ 3·23). (lit.[21] m.p. 285°). On treatment with diazomethane, *O*-methylcandicine was obtained, and isolated as the iodide, m.p. 209–210°. (lit.[22] m.p. 210–211°).

Tembetarine. It was isolated as the chloride and purified as described by Albónico *et al.*[9] M.p. 237–238°; $(\alpha)_D^{29}$ + 123·3° (*c.* 0·90, EtOH). Picrate, m.p. 190–191°.

N-*methylisocorydine.* The fractions containing this base were evaporated, the amorphous residue dissolved in a small amount of water and sodium styphnate solution added until precipitation was complete. The well-dried *styphnate* was dissolved in a small volume of acetone and filtered through a column of neutral alumina. The filtrate, on evaporation, yielded a crystalline residue which was recrystallized from ethanol giving long yellow prisms. M.p. 208–209°; $(\alpha)_D^{23}$ + 95·6° (*c.* 0·42, acetone). Found: C, 54·05; H, 4·78; N, 9·50; CH_3O, 15·59; $CH_3(N)$, 3·76; $C_{21}H_{26}NO_4.C_6H_2N_3O_8$ required: C, 54·01; H, 4·67; N, 9·34; CH_3O, 15·50; $CH_3(N)$, 5·00%). The styphnate was transformed into the iodide, m.p. 231–232°, $(\alpha)_D^{21}$ + 139·1° (*c.* 0·15, water) which on treatment with diazomethane, afforded di-*O*,*N*-methylisocorydine iodide, m.p. 250–251°; $(\alpha)_D^{23}$ + 167·3°, all in agreement with the data of Comin and Deulofeu.[6]

Magnoflorine. The styphnate was obtained by a similar procedure, m.p. 245–246°, $(\alpha)_D^{30}$ + 124° (*c.* 0·5, acetone). Iodide, m.p. 264°; $(\alpha)_D^{22}$ + 221·8° (*c.* 0·30, MeOH). Both salts were compared and found identical to authentic samples.[23]

Fagara naranjillo

Tembetarine and magnoflorine. The isolation of tembetarine chloride from specimens which contain only that base has already been described.[9]

In the residue (C) from *F. naranjillo* var. *paraguariensis*, small amounts of magnoflorine could also be detected. The two bases were separated by chromatography on a column of cellulose, crystallized as salts and identified as described for *F. rhoifolia.*

Fagara coco

Candicine, N-*methylisocorydine, and magnoflorine.* Because of the presence of tertiary bases in the bark of this species the acid solution (A) was made alkaline with ammonia (pH 8) and extracted with ether, which also dissolved the benzophenanthridine alkaloids. Paper chromatography of the ether extract revealed the presence of skimmianine, allocryptopine[7] and fagarine II.[5a]

The extracted aqueous solution was then acidified to pH 6 with 1 N HCl, passed through the column of Amberlite and worked as usual. From the residue (C), candicine, *N*-methylisocorydine, and magnoflorine were easily separated and crystallized as salts.

[21] L. RETI, *Compt. Rend. Soc. Biol.* **114**, 811 (1933).
[22] T. NAKANO, *Chem. Pharm. Bull.* (*Tokyo*) **2**, 321 (1954).
[23] T. NAKANO, *Chem. Pharm. Bull.* (*Tokyo*) **2**, 329 (1954).

Fagara hyemalis

With this and the following species separtion of the bases from residue (C) by the method described presented difficulties, and good results were only obtained by submitting it to 200 transfers in a counter current distribution apparatus, with tubes containing 40 ml of each phase, and using the system: *n*-butanol 0·1 NHCl. The concentration of the alkaloids was determined by measuring the optical density at 283 nm. The bases present in the tubes after distribution were revealed by paper chromatography, and isolated as described below.

Bases A-82 (K=0·06). This fraction was reserved for further examination.

Coryneine (K=0·10). Both phases were evaporated to dryness and the residue, on the addition of methanol, crystallized easily as the chloride. Recrystallized from ethanol prisms were obtained, m.p. 260°, λ_{max}^{EtOH} 208 nm (log ϵ 3·94), 283·5 nm (log ϵ 3·47). Treatment with diazomethane produced di-*O*-methylcoryneine chloride, m.p. 204–205°, and with diazoethane the corresponding di-*O*-ethylcoryneine chloride, m.p. 123–124° in full agreement with the data of Buck *et al.*[24]

Candicine (K=0·17). It was worked as described for coryneine, and the crystalline chloride isolated.

Tembetarine, magnoflorine, and laurifoline (K=0·35). After evaporation of the two phases, the residue was dissolved in warm methanol when most of the tembetarine chloride crystallized out. The mother liquor was again evaporated to dryness and separated by chromatography on a column of cellulose (35 × 105 mm). The remaining tembetarine was eluted first and isolated as the chloride. The fractions containing the base next eluted were evaporated to dryness, and the alkaloid identified as magnoflorine by preparation of the styphnate.

The last base eluted was identified as laurifoline. It easily gave a crystalline chloride, which on recrystallization from methanol, had m.p. 257° (dec), $(\alpha)_D^{27}$ +25·3 (*c.* 0·94, water), λ_{max}^{EtOH} 227, 281, 306 nm (log ϵ 4·53, 4·03, 4·14); picrate, m.p. 225° (dec), in agreement with the data of Tomita *et al.*[25] On treatment with methyl iodide in N KOH (methanol) di-*O*-methyllaurifoline iodide, was obtained m.p. 222–223° $(\alpha)_D^{22}$ +82·5° (*c.* 0·59, MeOH), which was found identical with glaucine methiodide. Employing ethyl iodide the known di-*O*-ethyllaurifoline iodide, m.p. 223° was produced.[26]

Fagara nigrescens

Bases A-82 (K=0·06). This fraction was reserved for further examination.

Candicine (K=0·17) was identified as indicated before for *F. hyemalis*.

Tembetarine, N-methylcorydine, and methylisocorydine. (K=0·35) Paper chromatography revealed that the main alkaloid was tembetarine which, on evaporation of both phases and dissolution of the residue in warm methanol, separated as the chloride. The mother liquor was passed through a column of cellulose (48 × 570 mm). After elution of the tembetarine the following fraction contained N-*methylcorydine*. They were evaporated to dryness and the crystalline styphnate prepared; m.p. 206–207°, $(\alpha)_D^{22}$ +128·7° (*c.* 0·26, acetone). The full identification of this base has been described by Kuck.[10]

The last eluted base was crystallized as styphnate and identified as *N*-methylisocorydine.

Xanthoplanine (K=1.00). The central tubes, containing only one base, were evaporated to dryness and a crystalline styphnate prepared, which was purified by recrystallization from ethanol-ether; m.p. 216–217°, $(\alpha)_D^{20}$ +50·4° (*c.* 0·56, acetone); picrate m.p. 229–230°, $(\alpha)_D^{29}$ +50·3° (*c.* 0·73, acetone); iodide m.p. 207–209°, $(\alpha)_D^{21}$ +62·0° (*c.* 0·83, EtOH) in agreement with the values given by Ishii and Harada[27] for the same derivatives of the original xanthoplanine. As a further identification, *O*-ethylxanthoplanine iodide was prepared, m.p. 225–226°, $(\alpha)_D^{29}$ +76·3° (*c.* 0·66, MeOH). By a Hofmann reaction it gave only an oily methine, whose methiodide, m.p. 274–275°, was again submitted to a Hofmann degradation and a second crystalline methine isolated, m.p. 135–137°. Both methines were found to be identical to authentic samples, obtained by Rüegger,[28] from *N*-methyllaurotetanine.

Fagara pterota

Most of the laurifoline chloride crystallized spontaneously on concentration of the solution (B). Mother liquors were evaporated and the residue (C) submitted to counter current distribution. The peaks contained the following bases: Bases A-82 (K=0·06); candicine (K=0·17); tembetarine; magnoflorine, *N*-methylisocorydine, and laurifoline (K=0·35). The candicine chloride was crystallized and identified as usual. The tubes containing the four latter bases were evaporated and the residue on treatment with methanol gave crystalline tembetarine chloride. After evaporation of mother liquor the residue was chromatographed on cellulose (35 × 105 mm) and the bases eluted in the following order: tembetarine, *N*-methylisocorydine, magnoflorine, and laurifoline. They were identified as already described.

[24] J. S. Buck, R. Baltzly and W. S. Ide, *J. Am. Chem. Soc.* **60**, 1789 (1938).
[25] M. Tomita and F. Kusuda, *Chem. Pharm. Bull. (Tokyo)* **1**, 1 (1953).
[26] F. Kusuda, *Chem. Pharm. Bull. (Tokyo)* **1**, 55 (1953).
[27] H. Ishii and K. Harada, *J. Pharm. Soc. Japan*, **81**, 238 (1961); *Chem. Abstr.* **55**, 14495 (1961).
[28] A. Rüegger, *Helv. Chim. Acta* **42**, 754 (1959).

Fagara chiloperone

The residue (C) was submitted directly to counter current distribution. The following peaks were observed, bases A-82 (K = 0·06), candicine (K = 0·17), tembetarine, magnoflorine, and laurifoline (K = 0·35). They were isolated as crystalline salts and identified as described for *F. hyemalis*.

Separation and Identification of Berberine and Benzophenthridine Alkaloids

The aliquot fraction separated from the original acid solution (A) was extracted with ether and made alkaline with concentrated ammonia, when a slight precipitate was formed, which was eliminated by filtration From the filtrate, the benzophenanthridine bases were extracted with chloroform. The aqueous solution was then alkalinized to pH 13, with 4 N NaOH, and the berberine bases extracted with the same solvent.

On paper chromatography, the first chloroform extracts never showed the presence of more than two bases. They were evaporated to dryness, the residue was dissolved in the smallest possible amount of ethanol and nitric acid carefully added. The nitrates then precipitated in a crystalline condition. When only chelerythrine was present, as indicated by chromatography, the nitrate was recrystallized from ethanol, m.p. 246–247°, and identified by transformation into the chloride, m.p. 240° and the preparation of dihydrochelerythrine, m.p. 159–161°.[29] If nitidine nitrate was the only salt obtained, it was recrystallized from water, m.p. 269–270°, and identified by further transformation into the pseudocyanide, m.p. 236–237°, and the preparation of oxinitidine, m.p. 284–285°.[30]

When both bases were present in the extracts, a good separation was obtained by boiling the mixture of the crystalline nitrates with 25 parts of boiling ethanol, which dissolves the chelerythrine salt, while most of the nitidine nitrate remains insoluble. The separated salts were purified and identified as described.

In the case of *F. coco*, the second chloroform extracts, containing the berberine alkaloids, were concentrated to dryness and 20% acetic acid was added slowly to the residue, scratching the contents, until a crystalline precipitate containing both bases was formed. It was filtered, dried, suspended in 50 parts of water, and the suspension heated to boiling point and filtered. The filtrate contained the berberine acetate with traces of palmatine, while the insoluble acetate of the latter base was collected in the filter.

Berberine was identified by transformation into tetrahydroberberine, m.p. 169–170°,[31] and palmatine by preparation of the chloride, m.p. 199–210°,[32] iodide, m.p. 235–237° and of tetrahydropalmatine, m.p. 146–147°.[33]

[29] P. J. SCHEUER, M. Y. CHANG and C. E. SWANHOLM, *J. Org. Chem.* **27**, 1472 (1962).
[30] H. R. ARTHUR, W. H. HUI and Y. L. NG, *J. Chem. Soc.* 1840 (1959).
[31] E. SPÄTH and N. POLGAR, *Monatsh. Chem.* **52**, 117 (1929).
[32] K. FEIST and G. SANDSTEDE, *Arch. Pharm.* **256**, 1 (1918).
[33] K. FEIST, *Arch. Pharm.* **245**, 586 (1907).