according to the method of Torssell and Wahlberg.[1] Column chromatography first on silicic acid and next on neutral alumina yielded a purified alkaloid fraction.

Actinidine. TLC of the purified alkaloid fraction, or of the $CHCl_3$–MeOH extract on silica gel HF in $CHCl_3$–MeOH (5:1), or in EtOAC–*iso*-PrOH–NH_4OH, (45:35:20), yielded several Dragendorff's positive bands. Preparative TLC of the Dragendorff's positive band with the highest R_f, in hexane–acetone–Et_2NH (4:1:1), yielded a purified Dragendorff's positive band with the same retention time as that of authentic actinidine. GLC on a 3·6 m × 6 mm glass column, packed with 15% Carbowax 20 M on Anakrom ABS, using a modified Barber–Colman Model 5000 gas chromatograph[5] equipped with a hydrogen flame detector, yielded a symmetrical peak with the same retention time as that of authentic actinidine. Co-chromatography with authentic actinidine confirmed the identification. Mass spectra of the isolated compound was very similar to that of authentic actinidine. Major fragment ions found in the mass spectrum of this compound were M^+ 147, *m/e* 132 [M—CH_3], *m/e* 117 [M—CH_3—CH_3] and a metastable ion at *m/e* 103·8 confirming the transition *m/e* 132, *m/e* 117 + 15, which agrees with the published mass spectrum by Auda *et al.*[6]

Mass spectra. The prototype[5] of the LKB-9000 gas chromatograph-mass spectrometer, (LKB Instruments Incorporated, Rockville, Maryland), was used. The instrument was operated at 70 eV, 3·5 kV accelerating voltage, 20 μA trap current, 290° ion source temp., 160° column temp. and a helium flow rate of 20 ml min.

Acknowledgements—We thank Dr. C. F. Van Sumere for the dried *V. officinalis* roots, Dr. Takeo Sakan for a sample of authentic actinidine, and W. Springstube and J. Marshall for technical assistance.

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ZINGIBERACEAE

FLAVONOLS AND QUINONES IN STEMS OF *AFRAMOMUM GIGANTEUM*

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Abstract—Three rare naturally occurring flavonol 3-methyl ethers have been isolated from stems of *Aframomum giganteum*: kaempferol 3,7,4′-trimethyl ether, quercetin 3,7,4′-trimethyl ether (ayanin) and quercetin 3,7,3′,4′-tetramethyl ether (retusine). The following compounds have also been isolated: chrysophanol, physcion, 2,6-dimethoxybenzoquinone and β-sitosterol. Phytochemical aspects of the flavonoids occurring in the Zingiberaceae are discussed.

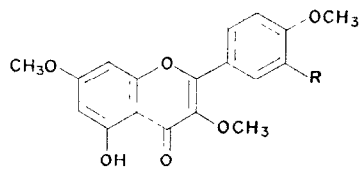
INTRODUCTION

Aframomum giganteum K. Schum. (syn. *Amomum giganteum* Oliv. and Hanb., *Zingiberaceae*), grows in the tropical zone of Central Africa. The plant reaches a height of 5–6 m and all the

parts have a very intensive and characteristic smell. Natives use stems, leaves, seeds and fruits for many purposes; in particular the plant seems to have healing properties. Few *Aframomum* species have so far been investigated and then only as far as the essential oils are concerned.[1,2] This is the first investigation on the pigments occurring in this genus.

RESULTS AND DISCUSSION

The stems have been successively extracted with hexane, $CHCl_3$ and 95% EtOH. From the first extract chrysophanol (1,8-dihydroxy-3-methylanthraquinone), physcion (1,8-dihydroxy-3-methoxy-6-methylanthraquinone), kaempferol 3,7,4′-trimethyl ether and quercetin 3,7,3′,4′-tetramethyl ether have been isolated. In the $CHCl_3$ extract the presence of 2,6-dimethoxybenzoquinone and quercetin 3,7,4′-trimethyl ether could be proved, whereas β-sitosterol was found in the last extract. The structures of all compounds were established by spectroscopic methods (IR, UV, NMR, MS) and confirmed by comparison with authentic samples. For chrysophanol,[3–5] physcion,[3,5] 2,6-dimethoxybenzoquinone[6] and β-sitosterol[7] data were identical with those reported in the literature. In the case of the three flavonol 3-methyl ethers, already known from other natural sources, literature data were lacking or did not agree with ours, possibly because recorded under different conditions. Therefore, we discuss briefly the assignment of their structures. Since the amounts isolated were insufficient for chemical methods to be used, we relied on spectroscopic evidence.



I R = H mol wt = 328
II: R = OCH_3 mol wt = 358
III: R = OH mol wt = 344

The UV spectra were indicative of the flavonol nucleus. The parent peak in the mass spectra, which is also always the base peak, gave the exact molecular weight for I, II and III. Bathochromic shifts (50–55 nm) in the UV spectra, when $AlCl_3$ was added,[8] together with the presence of a hydrogen bonded conjugated carbonyl group in the IR and of a hydroxyl shifted downfield owing to hydrogen bonding in the NMR spectrum, indicated a 5-hydroxyflavone system for all three compounds. The 3-methoxyl group in I–III was deduced from the benzene induced shifts of the OCH_3 resonances (see Table 1). Only one OCH_3 showed a small shift.[9] Furthermore the strong (M-43) cation in the mass spectra was ascribed to the loss of an acetyl radical from the 3-position.[10] Beside the 3-OMe, two other methoxyl groups were present in the NMR spectra of I and III, and three methoxyl groups in the NMR of II. The substitution pattern of the A ring was identical for the three

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TABLE 1. CHEMICAL SHIFTS OF PROTONS IN δ ppm

	A Ring*		B Ring*				5-OH*	OCH_3	
Compound	H-6	H-8	H-2′	H-6′	H-3′	H-5′		in $CDCl_3$	in C_6D_6
I	6·35†	6·45†	8·07‡		7·02‡		12·7§	3·87 (6H)¶ 3·89 (3H)¶	3·18 (3H)¶ 3·28 (3H)¶ 3·71 (3H)¶
II	6·35†	6·45†	7·69–7·85**		—	7·0††	12·64§	3·89 (6H)¶ 3·98 (6H)¶	3·21 (3H)¶ 3·40 (3H)¶ 3·52 (3H)¶ 3·78 (3H)¶
III	6·35†	6·45†	7·68–7·83**		—	6·98††	12·62§	3·89 (6H)¶ 4·00 (3H)¶	3·11 (3H)¶ 3·15 (3H)¶ 3·71 (3H)¶

* In $CDCl_3$, TMS=O.
† Doublet $J = 2{\cdot}5$ c/s.
‡ Doublet $J = 9$ c/s.
§ Singlet, disappearing with D_2O.
¶ singlet.
** multiplet.
†† doublet $J = 9{\cdot}5$ c/s.

flavonols as indicated by the correspondence in the NMR spectra, which showed an AB system of two aromatic protons (H-6 and H-8) with typical *meta*-coupling constants (centred on their chemical shifts) (see Table 1). The remaining four aromatic protons of compound I formed an A_2B_2 system, showing the presence of a *para*-substituted B ring, with the last OCH_3 in 4′. The spectral information was therefore sufficient to identify I as kaempferol 3,7,4′-trimethyl ether. In the NMR spectra of II and III the remaining three aromatic protons formed the characteristic pattern for a 3′,4′-disubstituted B ring (see Table 1). Structure of quercetin 3,7,3′,4′-tetramethyl ether (retusine) was assigned to II.

For compound III, the assignment of the last free hydroxyl group either to the A or to the B ring was made from the UV spectrum.[8] The absence of a shift in the 271 nm band on treatment with AcONa indicated that the 7-OH was protected by methylation. Furthermore no shift of the 357 nm band was observed by addition of fused AcONa to the methanolic solution while treatment with CH_3ONa produced a shift of only 33 nm and a lowering of the band intensity. These data were sufficient to exclude the 4′-position for the free OH group and to place it in the 3′-position. Therefore the structure of quercetin 3,7,4′-trimethyl ether was assigned to III.

CONCLUSIONS

The three flavonol 3-methyl ethers isolated from *Aframomum giganteum* are rare naturally occurring compounds. Previously each of them had been found only once in widely differing dicotyledonous families: I in the leaves of *Cheilanthes farinosa* (Polypodiaceae),[11] II in *Ariocarpus retusus* (Cactaceae)[12] and III in the heartwood of *Distemonanthus*

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benthamianus (Leguminosae).[13] Previously it was suggested that a characteristic feature of the flavonoids in the Zingiberaceae is the absence of a free hydroxyl group on the B ring.[14] Furthermore, of the kaempferol methyl ethers, only the 4′-methyl ether (kaempferide) has been found in the rhizome of *Alpinia officinarum*.[15] Recently kaempferol 7-methyl ether (rhamnocitrin) and kaempferol 3,7-dimethyl ether (kumatakenin) have been isolated from the seeds of *Alpinia japonica*[16] and of *Alpinia kumatake*.[17] In the last species, kaempferol and quercetin are also present. It is interesting to note that, while kaempferol methyl ethers seem at present to be restricted in the Zingiberaceae to *Alpinia* and *Aframomum* species, the quercetin methyl ethers have been hitherto found only in *A. giganteum*. A common feature of most of these derivatives, possibly of some taxonomic importance, is the presence of a 7-methoxyl group and of a free 5-hydroxyl group.

Quinones, never found before in the Zingiberaceae, are not as widespread in the monocotyledons as in the dicotyledons. Naphthoquinones have been isolated from the Iridales, and with anthraquinones from the Liliales.[14] Finally, it is noteworthy that chrysophanol, physcion, (widely occurring in the Polygonaceae and Rhamnaceae) and 2,6-dimethoxybenzoquinone have been found for the first time together in the same species, and to our knowledge, for the first time in the monocotyledons.

EXPERIMENTAL

Plant material. The stems of *Aframomum giganteum* collected in the Central African Republic was provided by Prof. M. Pavan, Institute of Entomology of Pavia. The identity of the species has been checked by Prof. R. Tomaselli, Botanical Institute of this University.

Isolation of compounds. 65 kg of stems were extracted by percolation with hexane. A second sample (4 kg) was also extracted with $CHCl_3$ and 95% EtOH.

From the hexane extract, after removing the volatile compounds by steam distillation and the fatty acids with 10% $NaHCO_3$, phenolic compounds were isolated with 5% NaOH. By silicic acid chromatography of the mixture (2·2 g) two coloured fractions were obtained. the former (A fraction), eluted with C_6H_6–$CHCl_3$ (1.1) developed a red colour by treatment with bases, the latter (B fraction), eluted with $CHCl_3$–ether (2:1) afforded two spots on TLC yellow brown in UV light, changing to yellow with bases.

By polyamide column chromatography of fraction A (eluant: EtOH–HOAc–HCO_2H, 5:1:1) two anthraquinones were separated: chrysophanol (22 mg, m.p. 194–195°) and physcion (5 mg, m.p. 205–207°) Preparative TLC of fraction B, successively eluted with C_6H_6–$CHCl_3$ (1:1) and with C_6H_6–$CHCl_3$–ether (5·5:1), afforded kaempferol 3,7,4′-trimethyl ether (I) and quercetin 3,7,3′,4′-tetramethyl ether (II). The residue of the $CHCl_3$ extract was chromatographed on silicic acid, 19 fractions were collected and monitored by TLC, only one fraction eluted with $CHCl_3$–CH_3OH (4:1) was examined. Treatment with isopropyl ether gave a gold yellow precipitate (2 mg), which was identified as 2,6-dimethoxybenzoquinone. Washings of the mother liquors with 5% NaOH yielded quercetin 3,7,4′-trimethyl ether (III) From the alcoholic extract β-sitosterol (11 mg) was obtained by routine separation.

Kaempferol 3,7,4′*-trimethyl ether* (I), 19 mg, m.p. 145–147° (lit.[11] 144–147° and 143–145°). ν_{max} (KBr) = 3440 (OH), 1655 (CO), 1600 and 1585 (C=C) cm^{-1}. λ_{max}^{EtOH} 269, 320 sh and 349 nm (log ε 3·82, 3·67; 3·75), shift with $AlCl_3$ 55 nm. NMR: see Table 1. MS (70 eV, DIS 170°): 328 (M^+, 100%), 327 (75%), 299 (M-CHO, 12%), 285 (M-CH_3CO, 49%), 150 (24%), 135 (23%)

Quercetin 3,7,3′,4′*-tetramethyl ether* (*retusine*) (II), 15 mg; m.p. 158–160° (lit.[12] 160–161°). ν_{max} (KBr) = 3440 (OH), 1655 (CO), 1607 and 1580 (C=C) cm^{-1}. λ_{max}^{EtOH} 213, 255, 270, and 356 nm (log ε 4 52; 4·28; 4 21; 4 24); shift with $AlCl_3$ 50 nm NMR: see Table 1. MS (70 eV; DIS 250°): 358 (M^+, 100%), 357 (57%), 343 (M-CH_3, 53%) 327 (M-CH_3O, 19%), 315 (M-CH_3CO, 68%), 167 (18%), 165 (43%), 150 (18%), 149 (23%), 142 (16%), 136 (13%), 119 (16%).

Quercetin 3,7,4′*-trimethyl ether* (*ayanin*) (III), 2 mg; m.p. 174–175° (lit.[13] 172–173°); ν_{max} (KBr) = 3400

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(OH), 1650 (CO), 1600 and 1580 (C=C) cm^{-1}. NMR: see Table 1. λ_{max}^{MeOH} (log ϵ) 257 (4·37), 271 (4·29) and 357 (4·31) nm; + NaOMe, 268 and 390; + $AlCl_3$, 241 sh, 271, 280 sh, 302 sh and 410; + NaOAc, 257, 271 and 357 nm; MS (70 eV; DIS 100°): 344 (M^+, 100%), 343 (83%), 329 (M-CH_3, 13%), 301 (M-CH_3CO, 43%) 167 (15%), 158 (18%), 151 (11%).

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ZYGOPHYLLACEAE

L(−)-4-HYDROXYPIPECOLIC ACID FROM *PEGANUM HARMALA*

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Abstract—Pegaline, an amino acid from *Pegalum harmala*, has been identified as L(−)-4-hydroxypipecolic acid.

SIDDIQUI and Kemal reported[1] in 1964 the isolation of an amino acid m.p. 295–296° from the seeds of *Peganum harmala*, which they provisionally named pegaline. It analysed for $C_6H_{11}O_3N$, gave a yellow colour with ninhydrin characteristic of proline and a positive pine chip test. The presence of a secondary function was indicated by the formation of a nitroso compound on reaction with nitrous acid. On the basis of these observations the authors concluded that pegaline contains a five membered heterocyclic ring.

The present investigation of pegaline, $[\alpha]_D^{30}$ −14·6 (2% in H_2O), showed that the colour developed with ninhydrin depends greatly on the time and temperature of subsequent heating, and may appear as dull yellow to greyish green or purple.

The IR spectrum (Nujol) showed a strong band at 1605 cm^{-1} due to —COO^- and two peaks of medium intensity at 3120 and 3260 cm^{-1} due to >NH and —OH groups.

The NMR spectrum of pegaline in D_2O did not reveal any peak which may be ascribed to —OCH_3, >N-CH_3 or C-CH_3. The mass spectrum showed a molecular peak at 145, a $(M—COOH)^+$ peak at 100 but no $(M—CH_3)^+$ or $(M—CH_2OH)^+$ peaks. The latter peak is present, for example, in the ethyl ester of 4-hydroxymethyl proline.[2] On spraying with isatin in BuOH–EtOH, pegaline gave a dull green colour characteristic of piperidine derivatives and no intense blue colour like pyrollidine derivatives.[3] Pegaline thus appeared to contain a six membered heterocyclic ring instead of five membered ring.

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