FLAVONOIDS OF *GARCINIA KOLA* SEEDS

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Garcinia kola Heckel (Guttiferae), known in commerce as "bitter kola", is used extensively in African traditional medicine for the treatment of various diseases. In our investigations, the most common usage was found to be for the treatment of coughs and mouth infections. The plant is also used for the treatment of liver disorders. It is served in Nigerian homes to guests as an adjunct to the true kola nut. *Garcinia* species have been reported to be used as an aphrodisiac (1) and for the treatment of diarrhea and dysentery (2).

Earlier work on the phytochemistry of *Garcinia* species resulted in the isolation of triterpenes (3), xanthones (4), and biflavonoids (5,6) from various species. As part of a continuing project to study the constituents of plants used in Nigerian ethnomedicine, we have investigated the flavonoids of *G. kola* seeds.

Simple flavonoids: apigenin—5,7,4'-trimethylether (500 mg), apigenin —4'methylether (150 mg), and fisetin (3',4',7-trihydroxyflavonol) (280 mg) were isolated together with the biflavonoids: amentoflavone (5',8"-biapigenin) (500 mg), kolaflavanone I-3'-II-3-I-4'-II-4'-I-5-II-5-I-7-II-7-octahydroxy-II-3'-methoxy-3/8"-biflavanone (350 mg) and *GBI* II-3-I-4'-II-4'-I-5-II-5-I-7-II-7-heptahydroxy—3/8"-biflavanone (120 mg).

Although apigenin was not isolated in this work, flavonoids based on apigenin represent 60% of the total flavonoids present in the diethylether fraction of *G. kola* seeds. The biflavonoids isolated belong to both the GB-kolavanone and the amentoflavone series, both types have been isolated previously from the Guttiferae.

The use of the plant in the treatment of liver disorders could be attributed to the presence of biflavonoids in *G. kola* since complex flavonoids such as silybin are known antihepatotoxic agents. The isolated compounds are the major seed flavonoids of this plant, minor constituents are yet to be characterized and the constituents of the other fractions have not been investigated.

EXPERIMENTAL

PLANT MATERIAL.—Seeds of *G. kola* were bought from the local market at Nsukka and authenticated by our Department of Botany. A voucher specimen (UN/PHARM 080-OA) has been deposited in the Pharmacy Herbarium, University of Nigeria Nsukka.

EXTRACTION AND ISOLATION.—Air dried and peeled seeds of *G. kola* were milled to a coarse powder after removal of the soft brown testa. Seed powder (3.5 kg) was defatted with petroleum ether (bp 40–60°) and later extracted with acetone. The concentrated extract was digested successively with benzene (8 hrs.), diethylether (10 hrs.), and ethyl acetate (20 hrs.). The benzene and ethyl acetate fractions were set aside for further analysis. The diethylether fraction was chromatographed on a silica gel column; benzene, benzene-ethanol (various ratios) and ethanol were the eluants. The compounds were purified by preparative tlc (silica gel and cellulose) with *n*-butanol-water-acetic acid-acetone (7:1:1:2); toluene-ethyl acetate-formic acid (5:4:1) and 15% aqueous acetic acid as the solvent systems.

IDENTIFICATION OF FLAVONOIDS.—The flavonoid 5,7, dihydroxy-4'-methoxyflavone or 4'-methylapigenin was identified from uv analysis with shift reagents and nmr measurements and by direct chromatographic comparison with an authentic sample (from SIGMA, London). Fisetin (3',4',7 trihydroxyflavonol) was identified from its uv, ir, ms, nmr and co-tlc with an authentic sample (from SIGMA, London). 5,7,4'-Trimethylapigenin was characterized by correlation of the spectral data (uv, ir, ms and nmr) with those of other flavonoids (7,8) and co-tlc with the compound obtained from the methylation of apigenin. The identity of the biflavonoids were established from their uv, and ms spectra of the isolated parent compounds, the nmr data of the permethyl ethers and co-tlc with authentic samples.

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LITERATURE CITED

1. G. Harley, "Native African Medicine", Frank Cass and Company Ltd., London, (1970) p. 60 and 81.
2. J. R. Ainslie, "List of Plants Used in Native Medicine in Nigeria", Imperial Forestry Institute Oxford, (1937) p. 42.
3. R. T. Aplin, J. H. C. Blasdale, T. G. Halsall and G. M. Honby, *J. Chem. Soc., C* 246 (1967).
4. C. M. Rezende and O. R. Gottleib, *Biochem. Syst. Ecol.* I, II (1973).
5. P. G. Waterman and E. G. Crichton, *Phytochemistry*, **19**, 2723 (1980).
6. P. G. Waterman personal Communication (1981).
7. T. J. Mabry, K. R. Markham and M. B. Thomas, "The Systematic Identification of Flavonoids", Springer-Verlag, New York, (1970).
8. J. B. Harbourne, T. J. Mabry and H. Mabry, eds., "The Flavonoids", Chapman and Hall, London, (1975).

CONSTITUENTS OF *PITHECELLOBIUM MULTIFLORUM*

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Pithecellobium multiflorum Benth. (Fabaceae), known locally as "canafistula de boi", has shown strong uterine stimulating activity (stem bark, aqueous extract) (1), and a petroleum ether-soluble fraction of the roots showed ED_{50} 1.0 μg/ml in the P-388 lymphocytic system in cell culture (2). On fractionation, this activity was not observed and no active compounds could be isolated; lupeol and α-spinasterol were obtained.

EXPERIMENTAL

PLANT MATERIAL.—Roots of *Pithecellobium multiflorum* Benth. (Fabaceae) were collected in the Department of Loreto, Peru, in August, 1976. A sample is in the Herbarium of the National Arboretum, U.S.D.A., Washington, D.C.

ISOLATION.—Root material (1 kg) was extracted with light petroleum ether to afford a residue (0.73 g) which, when chromatographed on silica gel, (30 g) yielded lupeol (9 mg, 0.0009%), mp 213–214°, $[\alpha]^{25}D$ +31.2° (c 0.3, pyridine); [Lit. (3) mp 215°, $[\alpha]D$ +33°]; nmr, ($CDCl_3$) δ 1.68 (3H, s, 20-CH_3), 3.21 (1H, dd, J=6.2, 7.9 Hz, 3α-H) and 4.57, 4.67 (2H, bd s, 24-H_2); ms, m/z 426 (M^+, 100%), 218 (65), 189 (48), 135 (50), 121 (41), 109 (56), and 95 (49). The chromatography also yielded α-spinasterol (28 mg, 0.0028%), mp 168–169°, $[\alpha]^{25}D$ ±0° [Lit. (4) mp 166–168°, $[\alpha]^{25}D$ ±0°; nmr, ($CDCl_3$) δ 3.58 (1H, m, 3α-H) and 5.09 (3H, m, 7, 22 and 23-H); ms, m/z 412 (M^+, 100%), 300 (20), 271 (80), 255 (28), 246 (20) and 81 (33).

P. multiflorum had not been examined previously; *P. dulce* has also afforded lupeol and α-spinasterol, together with other triterpenes (4).

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LITERATURE CITED

1. G. S. G. Barros, F. J. A. Matos, J. E. V. Vieira, M. P. Sousa, and M. C. Medeiros, *J. Pharm. Pharmacol.*, **22**, 116 (1970).
2. R. I. Geran, N. H. Greenberg, M. M. McDonald, A. M. Schumacher and B. J. Abbott, *Cancer Chemother. Rep.*, **3** (2), 1 (1972).
3. Elsevier's Encyclopedia of Organic Chemistry, Vol. 14, Elsevier Publishing Co., 1940, *ibid.*, supplement, 1952.
4. S. K. Nigam and C. R. Mitra, *Ind. J. Chem.* 395 (1967).