Study of the Seed Oil of *Brachystegia nigerica* Hoyle and A.P.D. Jones

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The characteristics of *B. nigerica* and its fixed seed oil are reported. Fatty acid composition, as determined by gas-liquid chromatography, was: lauric, 0.24%; myristic, 0.45%; palmitic, 13.18%; stearic, 19.80%; oleic, 20.84%; linoleic, 43.65%; arachidic, 1.14%; and arachidonic, 0.90%. A study of the nonsaponifiable fraction showed it to be rich in sterols. Ergosterol and sitosterol were identified.

KEY WORDS: *Brachystegia nigerica*, ergosterol, fatty acids, physicochemical characteristics, seed oil, sitosterol.

Brachystegia nigerica Hoyle and A.P.D. Jones (Fam. Caesalpiniaceae) is a giant tree growing up to 30-m high, and is distributed wildly in Nigeria. The plant is popular in central parts of Anambra State of Nigeria, where it is known by the vernacular (Ibo) name *achi*. A few of the plants grow within residential areas and villagers tend to erect shrines beside them because of "powers" ascribed to the plants. The seed is used as a thickening agent in preparation of soups. Traditional healers purport to use extracts of the leaves and stem bark in treatment of stomach disorders, fevers and urinary tract infections, especially gonorrhea. A literature survey did not reveal any systematic investigation of this plant. We have undertaken a study of its nutritional and medicinal constituents. This paper, the first in the series, presents the results of a study of the seed oil of *B. nigerica*.

EXPERIMENTAL PROCEDURES

Materials. The plant parts were collected from Ajanu Maku, in Anambra State of Nigeria. The plant was identified and authenticated in the herbarium of the Forestry Research Institute of Nigeria (Ibadan), where voucher specimens were deposited. The seeds were air-dried and pulverized to a moderately coarse powder. Ergosterol, sitosterol and fatty acid methyl esters were obtained from Sigma Chemical Co. (London, U.K.).

Procedures. The air-dried seed powder was extracted with petroleum spirits (60–80°C) in a continuous extractor for 18 hr. Evaporation of the solvent under vacuum yielded the oil. Standard methods (1) were used to determine the physical and chemical characteristics of the oil. The oil was saponified, and the nonsaponifiable fraction (NSF) was separated. The mixed fatty acids were obtained from the aqueous layer, and the chemical characteristics were determined (1). The total dienes and monoenes of the mixed fatty acids were determined spectrophotometrically by the alkali isomerization method (2) and reference values of Hilditch *et al.* (3). The total of saturated fatty acids was determined by difference. The methyl esters of the fatty acids were obtained and analyzed on a Pye Unicam series 304 chromatograph (Pye Unicam, Cambridge, U.K.) as described by Obasi *et al.* (4).

Thin-layer chromatography (TLC) of the NSF was carried out on activated 0.25-mm silica gf 254 (Merck, Darmstadt, Germany), and the preparative layer chromatogram (P-TLC) employed a 1-mm layer of mixed indicator silica gel type Pf 254 + 366. The developing solvent was benzene/acetone (90:10, v/v). Sterols were visualized on TLC by spraying with the Liebermann-Burchard and Vanillin-H_2SO_4 reagents (5).

The sterols were identified by TLC by using reference ergosterol and sitosterol standards. A portion of the crystalline sterols (0.1 g) isolated from the P-TLC was acetylated by dissolving it in 0.5 mL acetic anhydride, followed by the addition of two drops of dry pyridine. The solution was heated for 10 min in a boiling water-bath and poured into 40 g of ice in a 100-mL conical flask covered with aluminum foil. This mixture stood at room temperature for 12–15 hr. The sterol acetates were taken up in chloroform and analyzed on a Pye Unicam series 304 chromatograph equipped with a 10% apiezon L column programmed from 100 to 200°C at 10°C per min while the nitrogen flow rate was 40 mL per min.

RESULTS AND DISCUSSION

The seeds of *B. nigerica* yielded 7.3% of fixed oil on a dry weight basis. The physicochemical characteristics of the oil are presented in Table 1.

Fifty grams of the oil yielded 41.9 g of mixed fatty acids, which gave an iodine number of 10.82 and saponifiable equivalent of 342.47. The total dienes, monoenes and saturated fatty acids in the mixed fatty acids were found to be 45.2, 20.1 and 34.7%, respectively. The constituent fatty acids are shown in Table 1.

TLC analysis of the NSF indicated the presence of sterols, as visualized with Liebermann-Burchard reagent.

TABLE 1

Physicochemical Characteristics and Fatty Acid Composition of Oil from Seeds of *B. nigerica*

Physicochemical characteristics	
Specific gravity (27°/27°C)	0.9423
Refractive index at 27°C	1.4641
Iodine value	9.79
Acid value	1.122
Saponification value	145.9
Nonsaponifiable fraction	14.4%
Fatty acid composition (%)	
Lauric	0.24
Myristic	0.45
Palmitic	13.18
Stearic	19.80
Oleic	20.84
Linoleic	43.65
Arachidic	1.14
Arachidonic	0.90

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The sterols isolated from the P-TLC bands were recrystallized in acetone and characterized by their melting points, 167–168.5°C for ergosterol and 136–137°C for sitosterol. Ergosterol was found to constitute up to 8.2% of the NSF while sitosterol was up to 12.5%. Gas liquid chromatography was used to confirm the identity of the sterol fractions with the aid of reference sitosterol and ergosterol acetates. The NSF of the fixed oil of *B. nigerica* is significant because of its high yield (14.4% of the oil). Many seed oils have been found to contain sitosterol. Ergosterol has been reported more frequently in animals and lower plants. The sterols of the red snail (*Arion empiricorium*) consist of up to 19–25% of ergosterol (6). It occurs in algae, bacteria, fungi and lichens (7–9). Yeast, which contains ergosterol up to 90–100% of the total sterols, provides a practical source of vitamin D (10). The search for ergosterol in higher plants has not been extensive; however, some fixed oils such as corn, peanut and linseed oils have been reported to contain it in relatively small quantities (11). The occurrence or ergosterol, a source of vitamin D, in *B. nigerica* is therefore noteworthy.

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