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(E)- and (Z)-Foeniculin, Constituents of the Leaf Oil of a New Chemovariety of *Clausena anisata*[1]

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Abstract

The leaf oils of *Clausena anisata* Hook. f. collected from Pokuase, Ghana, were found to contain (E)-foeniculin as a major constituent (>78%) in 17 of 19 samples. (E)-anethole was the major constituent of the two other samples (57% and 67%) with (E)-foeniculin as the second most important compound (38% and 29%). The identity of the new natural product (Z)-foeniculin, present in small amounts (0.2-1.4%), was based on a comparison of retention indices and mass spectra with those of a synthesized sample whose spectroscopic data, reported here for the first time, are in agreement with the proposed structure. Two other minor constituents were identified as the rarely encountered natural products dictagymnin (1-20%) and 4-(3-methyl-2-butenyl)oxybenzaldehyde (0-0.2%).

Key Word Index

Clausena anisata, Rutaceae, essential oil composition, chemotypes, foeniculin isomers, dictagymnin, photoisomerization.

Introduction

Clausena anisata is a medicinal plant widely used in West Africa for a variety of ailments and for its insecticidal properties. Its local Ghanaian name, Saman ndobir or Samandua, means "that which drives the ghosts or spirits away." Previously, we reported (1) that based on its leaf oil chemical composition, *C. anisata* growing in Ghana, Togo and Benin, can be classified into four major chemovarieties. Three of these gave high oil yields and were composed of either methyl chavicol (80-100%) (2,3), (E)-anethole (85-100%), found also in Indonesia (4), and sabinene (5) as the major constituents. The fourth variety gave low oil yields (< 0.05%) and was composed of up to 50 minor constituents. In contrast to the methyl chavicol and low yield varieties that were widespread throughout

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the three countries, the anethole variety was only found on the Ofankor plains in the Greater Accra region of Ghana.

We now report the discovery of a new chemical variety of the leaf oil of *C. anisata* composed of (E)-foeniculin as its major constituent in addition to a minor product, (Z)-foeniculin, which, to the best of our knowledge, has not been reported until now. These findings are of great importance considering the eventual commercialization of the oils of this plant.

Experimental

Plant Material and Oil Isolation: All plants collected were positively identified as *C. anisata* by Prof. Oteng-Yeboah and Mr. D. K. Abbiw of the Botany Department, University of Ghana, or by Mr. A. A. Enti of the Forestry Enterprises Limited, Accra, Ghana. Voucher specimens are deposited at the Ghana National Herbarium, Department of Botany, University of Ghana, Legon.

Fresh leaves of *C. anisata* collected in September 1995 and August 1996 from Pokuase village, 2 km northwest of Ofankor (the location of the (E)-anethole chemovariety), were hydrodistilled using the previously published procedure (1). Oils were obtained in 1% to 2 % yield. Batches of leaves from three individual plants in the same locality were extracted with petroleum ether (bp 40° to 60°).

GC and GC/MS: The oils, the petroleum ether extracts and the synthesized compounds were analyzed by both GC on a Hewlett-Packard 5890 gas chromatograph equipped with a polar Supelcowax 10 column (30 m x 0.25 mm x 0.25 μm) and a nonpolar DB-5 column (30 m x 0.25 mm x 0.25μm) and then by GC/MS using a Hewlett-Packard MSD 5972 mass spectrometer at 70 eV coupled to an HP 5890 GC equipped with a DB-5 column (30 m x 0.25 mm x 0.25 μm). The temperature program for the GC and the GC/MS was 40°C for 2 min, then 2°C/min to 210°C and this temperature held constant for 33 min. The identity of the components of the oil and extracts was based on their retention indices on both columns and their mass spectra.

NMR Spectroscopy: All spectra were recorded on a Bruker AM-300 spectrometer. The samples were run in deuterated chloroform at 300 K.

Photoisomerization: An oil sample of *C. anisata* containing greater than 95% (E)-foeniculin was dissolved in hexane and irradiated in a quartz tube with a medium pressure 450 watt mercury lamp for 6 h. The solution was then filtered to remove a yellowish solid and a GC analysis of the hexane layer was shown to be a mixture of (Z)- and (E)-foeniculin in a ratio of 5:1.

Synthesis of (Z)-Foeniculin: The synthesis involved an O-alkylation of p-hydroxybenzaldehyde (Williamson reaction) with 4-bromo-2-methyl-2-butene followed by a Wittig reaction of the alkylated benzaldehyde with ethyltriphenylphosphonium bromide.

Williamson Reaction: p-hydroxybenzaldehyde (1.01 g, 8.2 mmol) and 4-bromo-2-methylbut-2-ene (1.30 g, 8.8 mmol) were dissolved in 50 mL of anhydrous acetone. Potassium carbonate (1.14 g) was then added and the mixture was vigorously stirred and refluxed for 4 h. The reaction mixture was cooled to room temperature and 100 mL of water was added to dissolve the solid material. The aqueous phase was extracted three times with 50 mL of diethyl ether. The latter phase was washed twice with 0.1 N HCl, twice with water and 0.5 N NaOH, and finally three times with water. The organic phase was dried over anhydrous magnesium sulfate and filtered. Evaporation of the ether gave a yellowish oil. The 4-(3-methyl-2-butenyl)oxybenzaldehyde was purified by silica gel chromatography using the same conditions as described below for foeniculin and a yield of 65% was obtained as a pale yellow oil: MS, 70 eV, m/z(rel.int.): 123(11), 122(21), 121(53), 93(5), 69(96), 68(21), 67(15), 65(20), 53(13), 41(100); ^{1}H-NMR (300 MHz $CDCl_3$/TMS) : δ9.80(s, CHO), δ7.83(d, J=8.7, H-3,H-5), δ7.01(d, J=8.7, H-2, H-6), δ5.49(br t, H-8), d4.60(d, J=6.7, CH_2-O), δ1.81(s, CH_3), δ1.76(s, CH_3).

Wittig Reaction: 4-(3-methyl-2-butenyl)oxybenzaldehyde (190 mg, 1 mmol), ethyl-triphenylphosphonium bromide (576 mg, 1.5 mmol,) and potassium carbonate (1.5 mmol, 243 mg) were refluxed for 2 h in 2 mL of dioxane containing 30 μL of water. Water was then added to the reaction mixture and the aqueous phase was extracted three times with hexane. (Z)-foeniculin was then purified by flash

Table I. Percentage composition of the constituents of the oils of *Clausena anisata* from Pokuase, Ghana

Constituents	Retention indices		Foeniculin type[1]			(E)-anethole type[2]
	DB-5	Supelcowax	Min.	Max.	Mean	
limonene	1033	1184	0.00	0.55	0.16	0.09-0.13
β-phellandrene	1033	1190	0.00	1.62	0.73	0.94-1.16
methyl chavicol	1197	1653	0.00	0.22	0.02	0.44-0.49
(Z)-anethole	1259	1756	0.00	0.00	0.00	0.13-0.29
(E)-anethole	1294	1815	0.00	1.30	0.20	57.11-67.52
β-caryophyllene	1419	1569	0.00	0.66	0.25	0.15-0.20
α-humulene	1457	1640	0.00	0.73	0.28	0.22-0.31
germacrene D	1487	1685	0.00	0.48	0.16	0.13-0.22
dictagymnin*	1585	2077	1.32	19.76	3.61	0.53-0.83
(Z)-foeniculin	1642	2160	0.21	1.37	0.48	0.00-0.21
4-(3-methyl-2-butenyl) oxybenzaldehyde	1647	2416	0.00	0.23	0.12	0.00-0.00
(E)-foeniculin	1686	2238	78.75	97.00	93.15	29.25-38.18

1 = 17 samples; 2 = 2 samples

* = MS, m/z (rel.int.): 202(1.5), 135(10), 134(100), 133(70), 119(7.5), 117(8), 107(15), 91(9.5), 77(20), 69(40), 53(10.5), 41(67.5)

Table II. ^{1}H-NMR data of (Z)- and (E)- foeniculin*

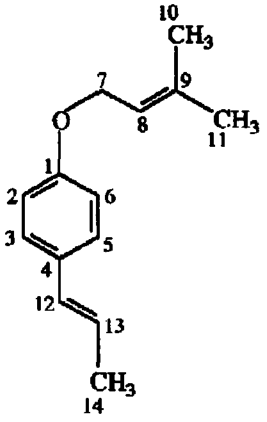
Proton	(Z)-Foeniculin	(E)-Foeniculin
H-3,5	7.24, d (8.7)	7.24, d (8.6)
H-2,6	6.90, d (8.7)	6.84, d (8.6)
H-12	6.36, d (11.6)	6.33, d (16.6)
H-13	5.69, m	6.06, m
H-8	5.50, br t (6.6)	5.48, br t (6.7)
H-7 (CH_2)	4.51, d (6.6)	4.48, d (6.7)
H-14 (CH_3)	1.88, dd (7.2, 1.8)	1.84, dd (6.6, 1.3)
H-11 (CH_3)	1.80, s	1.79, s
H-10 (CH_3)	1.74, s	1.73, s

*The chemical shifts are in ppm downfield from TMS and J values (in Hz) are in parentheses

chromatography followed by reverse phase HPLC as described below.

Chromatographic Purification of (Z)-Foeniculin: The mixture of foeniculin isomers (60 mg dissolved in pentane) was first separated by flash chromatography on silica gel using pentane:ethyl acetate, 97.5:2.5, as eluting solvent. Evaporation of the fractions rich in the foeniculin isomers gave 46 mg of a mixture of the *cis* and *trans* isomers in a ratio of 4:6 respectively as determined by capillary GC. This mixture was dissolved in methanol and then separated by reverse-phase HPLC using a semi-preparative LC-18 octadecylsilyl column (10 mm x 25 cm x 5 μm) and a UV detector at 254 nm. The mobile phase was methanol:water (75:25) with a flow rate of 6 mL/min. The (Z)-foeniculin (98% by GC) was eluted in 20 min and a quantity of 3.8 mg was obtained as a pale yellow oil: IR (GC/FTIR) ν_{max}, 3030, 2932, 1739, 1608, 1508, 1385, 1293, 1231, 1174, 1008, 838 cm^{-1}; MS, 70 eV, m/z(rel.int.): 202(3), 135(10), 134(100), 133(64), 119(6), 115(7), 107(10), 105(11), 91(9), 77(18), 69(30), 53(9), 41(55); ^{1}H-NMR (300 MHz $CDCl_3$/TMS): see Table II; ^{13}C-NMR (75.47 MHz $CDCl_3$/TMS): see Table III.

(E)-Foeniculin: Spectroscopic analysis was carried out on an oil fraction (pale yellow) composed of 98% (E)-foeniculin: IR (GC/FTIR) ν_{max}, 3043, 2935, 1606, 1507, 1375, 1276, 1232, 1169, 1007, 956, 838,



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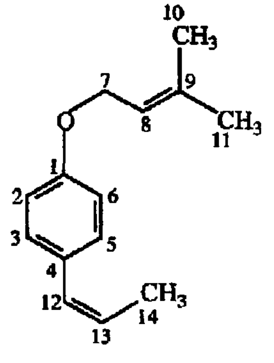

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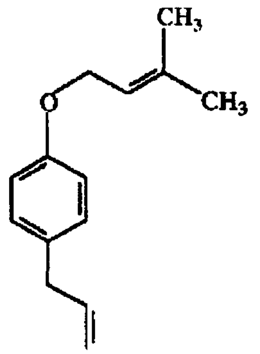

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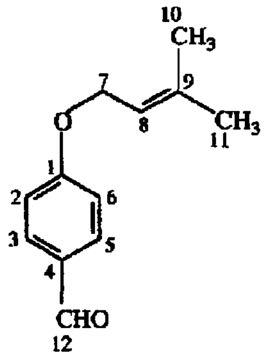

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786; MS, 70 eV, m/z (rel.int.) : 202(4), 135(10), 134(100), 133(63), 119(6), 115(7), 107(9), 105(9), 91(7), 77(17), 69(23), 53(9), 41(47); ^{1}H-NMR (300 MHz CDCl$_3$/TMS) : see Table II; ^{13}C-NMR (75.47 MHz CDCl$_3$/TMS): see Table III.

Results and Discussion

Oil Composition: The compounds identified in the leaf oils of *C. anisata* are listed in Table I. In 17 of 19 samples studied, (E)-foeniculin [**1**] was found to be the major constituent. In 15 of these 17 samples (E)-foeniculin (6,7) was present in amounts > 90%. The other two samples contained 78% and 86% of (E)-foeniculin. (E)-Anethole was the major constituent of the two other samples (57% and 67%) with (E)-foeniculin as the second most important compound (38% and 29%). The identity of the new natural product (Z)-foeniculin [**2**], present in small amounts (0.2-1.4%), was based on a comparison of retention indices and mass spectra with those of a sample obtained by photoisomerization of natural (E)-foeniculin and a sample obtained by synthesis, whose spectroscopic data, reported now for the first time, are in agreement with the proposed structure (Tables II and III).

It is unlikely that the *cis* isomer is an artifact formed during the steam distillation as in another experiment, the leaves of the plant were extracted at room temperature with hexane and the same

Table III. ^{13}C-NMR data of (Z)- and (E)- foeniculin*

Carbon	(Z)-Foeniculin	(E)-Foeniculin
1	157.45	157.84
9	138.10	137.78
4	130.29	130.66
3 and 5	129.98	126.76
12	129.35	130.39
13	125.02	123.18
8	119.80	119.80
2 and 6	114.33	114.59
7	64.79	64.68
11	25.83	25.69
10	18.20	18.30
14	14.60	18.06

* The chemical shifts are in ppm downfield from TMS

constituent was observed in the extract obtained in this manner.

Two other minor constituents were identified as the rarely encountered natural products dictagymnin (1-20%) [3] and 4-(3-methyl-2-butenyl)oxybenzaldehyde [4] (0-0.2%).

GC and GC/MS: The mass spectra of the foeniculin isomers were very similar. Their identities were first of all tentatively deduced by comparing their retention indices on a nonpolar column (Table I) with those of (E)- anethole, (Z)-anethole and methyl chavicol. As can be seen, the differences between the anethole retention indices are similar to the corresponding differences in the foeniculin isomers. The retention index on the polar column and mass spectrum of dictagymnin are in agreement with those previously reported (8). Some of these various isomers have been found together as constituents of other oils such as *Dictamnus albus* (8), *Foeniculum vulgare* (9) and *Illicium verum* (10). The identity of the minor constituent 4-(3-methyl-2-butenyl)oxybenzaldehyde was based on a comparison of its retention index and mass and ^{1}H-NMR spectra with those of an authentic sample synthesized by a Williamson reaction involving an O-alkylation of p-hydroxybenzaldehyde. This compound was reported for the first time as a natural product by Ayer and Trifonov (11) who isolated it from a culture broth of the fungus *Peniophora polygonia*.

Photoisomerization: A hexane solution of an oil sample of *C. anisata* containing greater than 95% (E)-foeniculin was irradiated with a medium pressure mercury lamp for 6 h. The extent of photoisomerization was followed by GC and the peak of the (E)- isomer was observed to decrease while the peak identified as the (Z)- isomer increased. GC analysis showed a ratio of 5 to 1 for the (Z)- and (E)- isomers respectively. *cis-trans* Photoisomerization of Z,Z-1,4-bis(1-propenyl)benzene has been studied previously (12).

NMR Results: The ^{1}H- and ^{13}C-NMR chemical shifts for the (E)- and (Z)- foeniculins are shown in Tables II and III. As can be seen from the data, both the proton and carbon spectra of the two isomers are very similar. The chemical shifts are in agreement with calculated values (13). The most important spectral information for the identification of the (Z)-foeniculin is the coupling constant of 11.6 Hz for the double bond hydrogen at C_{12} of the propenyl side chain which is consistant with the *cis* configuration. The corresponding coupling constant observed for the *trans* isomer is 16.6 Hz. Spin decoupling experiments of the proton NMR were also carried out and confirmed some of the chemical shift assignments. Irradiation of the methylene protons of carbon-7 reduced the broad triplet of H-8 to a singlet, irradiation of the H-12 signal transformed the H-13 multiplet to a quartet and the C-14 methyl from a doublet of doublets to a doublet, and finally irradiation of the C-14 methyl changed the H-13 multiplet to a doublet. These NMR experiments confirm the structure of (Z)-foeniculin.

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