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STERIODS CONSTITUENTS OF *DIOCLEA REFLEXA* HOOK SEEDS

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ABSTRACT
Plants are stouted as nutritionally useful foods which supposedly prevent cancers and provide other health benefits. Two steroids, Taraxasterol and Stigmasterol were isolated from the methanolic extract of the seeds of *Dioclea reflexa*. The structure elucidation of the compounds was performed by spectroscopic analysis and comparison with published data from literature.
KEYWORDS: Phytosterols, *Dioclea reflexa*, taraxasterol, stigmasterol, cancers

INTRODUCTION

Higher plants are known to provide a diverse range of secondary metabolites. The species *D. reflexa* Hook ("Agbaarin" in Yoruba) (Fabaceae) reproduces from seeds[1]. Some part in Nigeria majorly Yoruba land desire spice element in the seeds. The powdered cotyledon is used in preparing soup. It is valid because of it's swear aroma and sharp taste that increase appetite. The Igbos also used it for soup as soup thickner. The seed powder of *D. reflexa*, in Senegal is used in preparation with Palm oil to cure rheumatism and itching. The powdered seed is also used in Congo for treating cough. A roasted seed ground and mixed with kaolin is used orally against asthma[2].

The only previous phytochemical study reported in this species reported the anticholestarase and antibacterial activities of dioclimidazole from *D. reflexa* seeds[3]. In our previous work on the species, we reported the extraction and characterization of the seed oils of *D. reflexa*[4]. As part of a continuing search on native plants from Nigeria aimed at the characterization of poorly studied and identification of bioactive constituents, the methanolic extract of the seeds of *D. reflexa* yielded Taraxasterol **1** and stigmasterol **2**. Continuing with the phytochemical investigation of *D. reflexa*, this paper reports now its steroids composition.

MATERIALS AND METHODS

Plant material

The seeds of *D. reflexa* Hook were locally obtained in Ado-Ekiti, Ekiti State, Nigeria. It was botanically identified by Mr. F.O Omotayo of the Herberium Section, Department of Botany, Ekiti State University, Ado-Ekiti, Nigeria. The hard seeds were broken to remove the cotyledons which were further broken and made into a reasonable fine powder in a mortal with pestle.

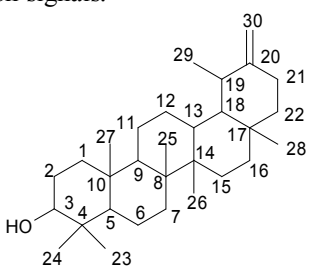
EXTRACTION AND ISOLATION

The fine powder of the seeds of *D. reflexa* was extracted with methanol for 72h at room temperature. Extraction was further repeated with methanol. The combined extract was concentrated using rotary evaporator to obtain a brownish coloured solid (9.49g) used for further fractionation. The residue (9.49g) was fractionated on silica gel open column chromatography eluted with an increasing gradient of ethylacetate in *n*-hexane up to 100%, followed by an increasing gradient of methanol in ethylacetate up to 100%. This gave four pooled fractions A-D. Fraction B (0.98g) was further fractionated on silica gel open column and eluted with an increasing gradient of ethylacetate in *n*-hexane up to 100% followed by an increasing gradient of methanol in ethylacetate up to 100%. This column yielded bioactive compounds Taraxasterol (0.18g) and stigmasterol (0.04g).

RESULT AND DISCUSSION

The methanolic extract of the *D. reflexa* seed, after successive column chromatography on silica gel, afforded taraxasterol and stigmasterol. These compounds were identified by comparison of their spectra data with those reported in the literature.

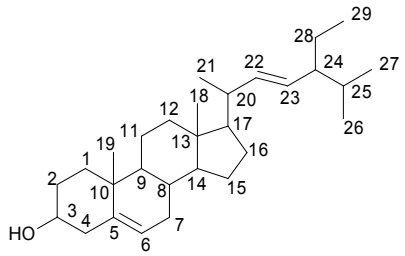
Table 1 shows the ^{13}CNMR spectrum of taraxasterol in comparison with one obtained from the root of *Cynara cardunculus*[5]. The ^{1}HNMR of compound **1** revealed 7 methyl signals with one of the methyl signal doublet, a quitet at δ 1.9, multiplet at δ 2.35, a quartet at δ 3.2 and a doublet at δ 4.6. These proton signals suggested a terpenoid or isoprenoid structure. The ^{13}CNMR showed 30 carbon atoms comprising of 7 methyl, 11 methylene, 5 methine and 6 quaternary carbon signals.



[**1**]

The down field region of the ^{13}CNMR spectrum revealed a quaternary carbon signal at δ151.2 and germinal methylene carbon signal at δ109.6. This pattern is supported by the carbon-29 methyl, hence, the structure of compound **1** is confirmed to be taraxasterol instead of lupeol, because for lupeol, all the methyl signals must be singlet since they all attached to quaternary carbon atoms. Both ^{1}HNMR and ^{13}CNMR signals were assigned by comparison with reported data for taraxasterol.

Table 2 shows the comparison of ^{13}CNMR spectra data of compound **2** with reported data obtained for stigmasterol[6]. The proton NMR spectrum for compound **2** revealed methyl signals between δ 0.62 to 1.5. A multiplet at δ 3.5, 5.1 and a doublet at δ 5.35. The ^{13}CNMR revealed 29 carbon atoms, 6 methyl, 9 methylene, 11 methine and 3 quaternary carbon signals. The down field region of the 13CNMR revealed a quaternary carbon signal at δ 141 and methane carbon signals at δ 138.5, 129.4 and 121.9 respectively. All the carbon signals compared very well with that reported for stigmasterol[6].



[**2**]

Stigmasterol has been reported to prevent certain cancers including ovarian, prostate, breast and colon cancers[7]. It also possessed antioxidant, hypoglycemic and thyroid inhibiting properties[8]. Tarasterol has been found to be a valuable chemopreventing agent against chemical carcinogenesis and anti-inflammatory agent[9]. These sterols have also been reported to have cholesterol lowering abilities. *Dioclea reflexa* can be used as preventive medicine for the curing of cancers. It can also be used to prevent accumulation of cholesterol in the body since it has been found to decrease the absorption of cholesterol and sitosterol that cause high blood pressure and other heart diseases.

CONCLUSION

This study therefore provided bases to the folkloric use of the seeds of *Dioclea reflexa* as a remedy for treating rheumatism, itching and other infections caused by pathogens. It also justifies the folklore medicinal uses and claims about the therapeutic values of this plant as curative agent. Research work is still on to further purify and characterize the phytochemicals from the plant with a view to obtaining more useful chemotherapeutic agents.

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Table 1: ^{13}C NMR spectra data for compound **1**

No	DEPT	**1**(δ ppm)	* δ_c ppm
1	CH_2	38.95	
2	CH_2	27.5	27.4
3	CHOH	79.2	79.1
4	C	39.0	38.8
5	CH	55.4	55.3
6	CH_2	18.5	18.3
7	CH_2	34.5	34.3
8	C	41.1	41.0
9	CH	50.7	50.5
10	C	38.3	37.3
11	CH_2	21.2	21.0
12	CH_2	25.4	25.2
13	CH	37.4	37.2
14	C	43.2	42.8
15	CH_2	27.2	27.1
16	CH_2	28.2	29.2
17	C	48.2	47.9
18	CH	48.5	47.9
19	CH	48.5	48.8
20	C	151.2	150.5
21	CH_2	29.9	29.8
22	CH_2	35.8	34.1
23	CH_3	27.7	28.1
24	CH_3	15.5	15.4
25	CH_3	16.0	16.1
26	CH_3	16.0	16.1
27	CH_3	14.8	14.8
28	CH_3	13.2	18.3
29	CH_3	19.5	19.1
30	CH_2	109.5	109.7

*[5]

Table 2. ^{13}CNMR spectra data for compound **2**

No	DEPT	**2** (δ ppm)	* δ ppm
1	CH_2	37.5	37.3
2	CH_2	31.9	31.7
3	CH	72.0	71.8
4	CH_2	42.5	42.4
5	C	141.0	140.8
6	CH	121.9	121.7
7	CH_2	31.9	31.9
8	CH	32.1	31.9
9	CH	50.4	50.2
10	C	36.7	36.6
11	CH_2	21.3	21.1
12	CH_2	39.9	39.7
13	C	42.5	42.4
14	CH	57.0	56.9
15	CH_2	24.5	24.4
16	CH_2	28.5	29.0
17	CH	56.2	56.1
18	CH_3	12.1	12.1
19	CH_3	19.6	19.4
20	CH	40.7	40.6
21	CH_3	21.2	21.1
22	CH	138.5	138.4
23	CH	129.5	129.3
24	CH	51.5	51.3
25	CH	32.1	31.9
26	CH_3	21.3	21.3
27	CH_3	21.2	19.0
28	CH_2	25.6	25.4
29	CH_3	12.3	12.3

*[6]

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