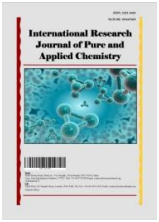

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Isolation and Characterization of an Amide, (2S)2-Hydroxy-N-((3R,4R)-1,3,4-Trihydroxytridecan-2-YL)Undecamide, from the Root Bark of *Ficus exasperata* (Vahl)

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Authors' contributions

This work was carried out in collaboration among all authors. Author OEF designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors EAA and HOO managed the analyses of the study. Author AO managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Silica gel (70-230 mesh ASTM) was packed into a column (45 cm × 3 cm) using the dry method. About 7 g of extract was mixed with 30 g of silica gel and allowed to dry. It was then loaded onto the column and successively eluted initially with pet ether 100%, followed by 10%, 20%, 40%, 60%, 80% chloroform in pet-ether; followed by 100% chloroform; 20%, 50% ethyl acetate in chloroform; followed by 100% ethyl acetate and then 20% and 50% methanol in ethyl acetate. 25 fractions were collected in 50 ml aliquots and bulked together according to their TLC profiles and R_f. The bulked fraction was further column chromatographed over silica gel (70-230 mesh) using a pipette and isocratically eluting with pet-ether: chloroform: methanol 67:25:8. 30 fractions of 2 ml each were collected. Compound C-3 (200 mg) was obtained from the fractions 1-15 as an off-white amorphous powder. The combination of IR, ^{1}HNMR, ^{13}CNMR, gCOSY, HMBC, HSQC and Mass spectral data on this off-white powder has led to an unambiguous assignment and the compound,

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an amide, isolated from the bioactive fraction of *F. exasperata* has the chemical name (2S)2-hydroxy-N-((3R,4R)-1,3,4-trihydroxytridecan-2-yl)undecamide. The acclaimed medicinal uses of this plant such as antihypertensive, anti-inflammatory, anti-arthritic, anti-ulcerogenic, anti-microbial and anti-oxidant, among others made it attractive to the authors.

Keywords: Ficus exasperate; amide; isolation; characterization; chromatography.

1. INTRODUCTION

Medicinal plants are plants which contain substances that could be used for therapeutic purposes or which are precursors for the synthesis of useful drugs [1]. Medicinal plants, since time immemorial have been used in virtually all cultures as a source of medicine. The widespread use of herbal remedies and healthcare preparations, as those described in ancient and holy texts such as Vedas and the Bible, and obtained from commonly used traditional herbs and medicinal plants, has been traced to the occurrence of natural products with medicinal properties. The use of traditional medicine and medicinal plants in most developing countries, as a normative basis for the maintenance of good health, has been widely observed [2]. Furthermore, an increasing reliance on the use of medicinal plants in the industrialized countries has been traced to the extraction and development of several drugs and chemotherapeutics from these plants as well as from traditionally used rural herbal remedies [3]. Moreover, in these societies, herbal remedies have become more popular in the treatment of minor ailments, and also on account of the increasing costs of personal health maintenance. Indeed, the market and public demand has been so great that there is a great risk that many medicinal plants today face either extinction or loss of genetic diversity. Medicinal plant drugs can be placed into two broad categories. Firstly, they are included in complex mixtures containing a wide variety of compounds (e.g. infusions, essential oils, tinctures or extracts), and secondly they are used as pure, chemically defined active principles [4,5]. Medicinal plants typically contain mixtures of different chemical compounds that may act individually, additively or in synergy to improve health. A single plant may, for example, contain bitter substances that stimulate digestion, anti-inflammatory compounds that reduce swelling and pain, phenolic compounds that can act as antioxidants and venotonics, antibacterial and antifungal tannins that act as natural antibiotics, diuretic substances that enhance the elimination of waste products and toxins and alkaloids that enhance mood and give a sense of well-being [6]. Many reasons have been given on why people use medicinal plants as therapy. Many plants are believed to be more effective than the orthodox medicines. There is also the preference of consumers for natural therapies, a greater interest in alternative medicines and a commonly held erroneous belief that herbal products are superior to synthetic products. In some African communities, traditional medicines are used because they are thought to help clean out negative spiritual influences [7]. Furthermore, the dissatisfaction with the results from synthetic drugs and the belief that herbal medicine might be more effective in the treatment of certain diseases where conventional therapies and medicines have proven to be ineffective. More so, many people turn to medicinal plant treatment in some developing nations because professional care (orthodox medicine) is not immediately available, too inconvenient, costly, or time consuming [8]. In rural areas, there are additional cultural factors that encourage the use of botanicals, such as the concept of an interplay between the environment and culture, a “man-earth” relationship [9]. The improvements in the quality, efficacy, and safety of herbal medicines with development of science and technology have also been largely responsible for the increase use of medicinal plants. Some patients also believed that their physicians have not properly identified the problem: hence they feel that herbal remedies might be another option [10].

The genus *Ficus* consists of woody trees, shrubs, vines, epiphytes, and hemiepiphytes [11]. They are collectively known as fig trees or figs. They are native throughout the tropics with few species extending into the semi-warm temperate zones. It is a deciduous tree with smooth gray bark and very rough (Scabrous) leaves. It is known by the common names “sand paper tree, “Ewe ipin” in Yoruba, ”ɛsasa mkuyu” in Swahili, “Papier de verre” in French, and in Ghana it is called “onyankyeren” (Akans), [12].

Ficus exasperata is a tree or shrub which can grow to about 20 m tall. The leaves alternate and have a scabrous upper surface. The lamina is ovate to elliptic or obovate. The apex is shortly acuminate and sometimes acute or obtuse. The

base is cuneate or occasionally subcordate and the margin dentate to sub entire. The fruits occur in pairs or solitary in the leaf axils, just below the leaves. The unripe fruit is green in colour, and about 8–15 mm in diameter. The fruits are orange in colour when ripe [13]. The bark is smooth, grayish cream with brown streaks and exudes a gummy sap.

2. MATERIALS AND METHODS

2.1 Materials

The solvents used for extraction, column chromatography and Thin Layer Chromatography analysis were of analytical grade and included methanol, ethyl acetate, chloroform, and pet-ether. The organic solvents and anisaldehyde were purchased from ROVET Scientific Limited, Osogbo.

2.2 Collection and Authentication of Plant Samples

The roots of *Ficus exasperata* were collected in a plantation in Ondo, Ondo State, Nigeria. The samples were identified at the Department of Crop, Soil and Pest Management, Federal University of Technology, Akure where voucher specimen has been deposited in the herbarium (CSPH2614).

2.3 Processing of Plant Materials

The root bark of *F. exasperata* was sun dried for 48 hours followed by oven drying at 40°C for further 48 hours. The material, thus dried, was coarsely milled and packed into brown paper bags and kept in the laboratory until required for use.

2.4 Extraction of Plant Materials

Preliminary extraction of 500 g of the coarsely powdered root bark of *F. exasperata* was done by soxhlet extraction using chloroform for 72 hours and concentrated to give the extract, CFE (yield value = 0.5% W/W). The extract was used for both thin layer and column chromatography before characterization.

2.5 Column Chromatographic Fractionation

Silica gel (70-230 mesh ASTM) was packed into a column (45 cm × 3 cm) using the dry method. About 7 g of extract was mixed with 30 g of silica gel and allowed to dry. It was then loaded onto the column and successively eluted initially with pet ether 100%, followed by 10%, 20%, 40%, 60%, 80% chloroform in pet-ether; followed by 100% chloroform; 20%, 50% ethyl acetate in chloroform; followed by 100% ethyl acetate and then 20% and 50% methanol in ethyl acetate. 25 fractions were collected in 50 ml aliquots and bulked together according to their TLC profiles and R_f.

The bulked fraction was further column chromatographed over silica gel (70-230 mesh) using a pipette and isocratically eluting with pet-ether: chloroform: methanol 67:25:8. 30 fractions of 2 ml each were collected. Compound C-3 (200 mg) was obtained from the fractions 1-15 as an off-white amorphous powder.

3. RESULTS AND DISCUSSION

3.1 Identification of Compound C-3

3.1.1 NMR interpretation of C-3

C-3 was obtained as an off white amorphous powder. the ^{13}C-NMR spectrum ((figure) exhibited 24 carbon resonances including two methyl, seventeen methylene, four methines, and one carbonyl carbon. the ^{1}H-NMR spectrum (Fig. 1) showed six methyl protons, thirty four methylene protons and four methine protons. the four hydroxyl groups present are in rapid exchange with the deuterium from the deuterated methanol used and peaks between 4.5 and 5.0. 3.0 and 3.5 in the proton NMR are solvent peaks (deuterated methanol). the amide group presence is supported by carbon shifts seen between 170 and 180 which are typical of carbonyl carbons present in amides. This is more visible in the HMBC which shows coupling between carbon and protons separated by two or more bonds. From HSQC, all the methine protons appear relatively downfield (between 3.5 and 5.8), this shows that they are attached to carbons attached to electronegative elements like O and N. All methylene protons are seen between 1.0 and 2.5 with 32 of them (1.0 to 2.0) of them not directly attached to an electronegative element. Only two of the methylene protons are directly attached to an electronegative element which is one of the OHs, this is the most downfield of the methylenes. from COSY, the methyl protons are directly attached to the most upfield four methylene protons. three methane protons (between 5 and 6) are directly attached to OH based on the very close chemical shifts of the methyl and

methylene protons, the structure of the molecule can be resolved as a symmetrical molecule with two almost identical parts separated by a moiety with electron withdrawing atoms like the amide group.

3.1.2 IR absorption data of compound C-3

As can be seen in Table 1, the functional groups present in isolate C-3 as revealed by the IR absorption data are; methyl, methylene, alcohol, carboxyl and amide.

3.2 Mass Spectral Data of C-3

ESI-MS in sodium matrix gave m/z 455 = [M+23] and 432×2+23 = 887 with actual molecular weight 431 and molecular formula $C_{24}H_{49}NO_5$.

The combination of IR, ^{1}HNMR, ^{13}CNMR, gCOSY, HMBC, HSQC and Mass spectral data has led to an unambiguous assignment and the compound isolated from the bioactive fraction of *F. exasperata* has the above chemical structure (Fig. 1).

Table 1. IR absorption data of compound C-3 from *F. exasperate*

Wave number cm^{-1}	Inference
1375	Amide
1452	C-H bending
1631.292	Amide N-H bending
1710.808	Amide C=O stretch
2851.434	C-H stretch
2919.88	C-H stretch
3400.123	OH stretch, H bonded
3512.402	Amide NH stretch

To our knowledge, this is the first report of isolation of this new bioactive amide from *F. exasperata* with the chemical name (2S)2-hydroxy-N-((3R,4R)-1,3,4-trihydroxytridecan-2-yl)undecamide. Table 2 shows the proton and carbon chemical shifts assignment using the drawn structure above (Fig. 1).

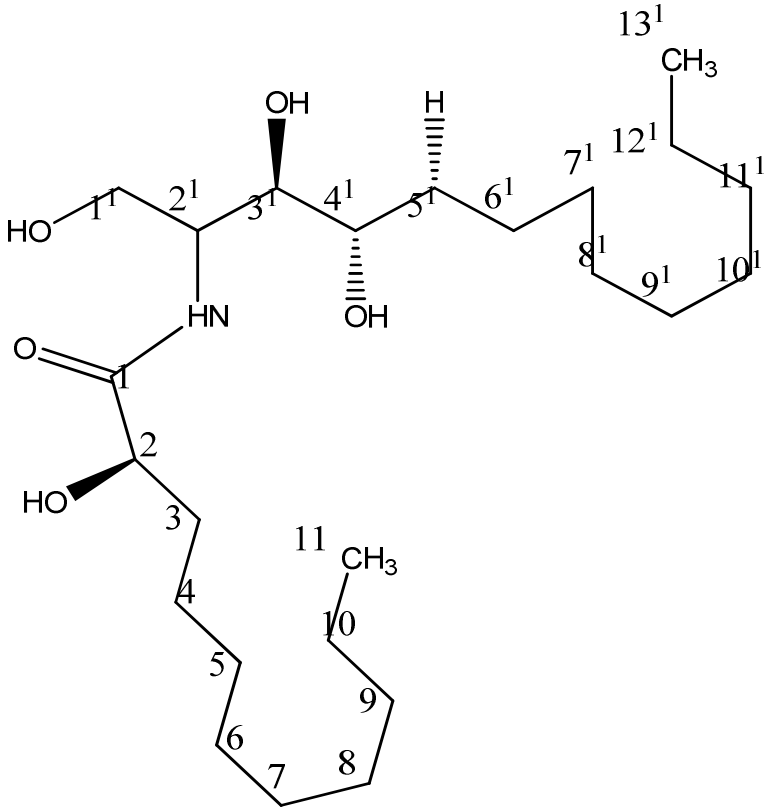

Fig. 1. Chemical structure of compound C-3 (2S)2-hydroxy-N-((3R,4R)-1,3,4-trihydroxytridecan-2-yl)undecamide

Table 2. ^{13}C and ^{1}H-NMR chemical shifts (ppm) for the new compound C-3

Position	Type	δ H	^{13}C	Position	Type	δ H	^{13}C
C-1	C=O	-	1.77	C-2[1]	CH	3.75; 8.03(NH)	57.3
C-2	CH	4.16; 2.80(OH)	73.1	C-3[1]	CH	3.91; 3.58(OH)	75.6
C-3	CH_2	1.72	35.9	C-4[1]	CH	3.29; 3.58(OH)	71.4
C-4	CH_2	1.25	27.5	C-5[1]	CH_2	1.44;1.44	33
C-5	CH_2	1.25	29.2	C-6[1]	CH_2	1.25	25.7
C-6	CH_2	1.29	29.6	C-7[1]	CH_2	1.25	29.9
C-7	CH_2	1.26	29.6	C-8[1]	CH_2	1.29	29.6
C-8	CH_2	1.29	29.3	C-9[1]	CH_2	1.26	29.6
C-9	CH_2	1.29	31.9	C-10[1]	CH_2	1.29	29.3
C-10	CH_2	1.31	22.7	C-11[1]	CH_2	1.29	31.9
C-11	CH_3	0.88	14.1	C-12[1]	CH_2	1.31	22.7
C-1[1]	CH_2	3.50;3.25; 3.65(OH)	61.4	C-13[1]	CH_3	0.88	14.1

4. CONCLUSION

The acclaimed medicinal uses of this plant led the authors to investigate the active constituents present in this plant. With the isolation of this novel amide, to our knowledge, it may be the compound responsible for the aforementioned medicinal uses. Further work will be done by the authors, in due course, to investigate the bioactivity of this amide and also isolate and characterize other active chemical compounds in the plant that may be synergistically responsible for its medicinal uses.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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