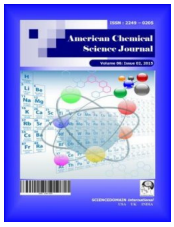

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Synthesis and Characterization of Novel Xylopic Acid Derivatives

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Authors' contributions

This work was carried out in collaboration between all authors. Author WK designed and directed the study and corrected the manuscript. Author JPF carried out the experimental work, wrote the protocol, and wrote the first draft of the manuscript. Authors WK and RKA managed the study. Author JPF managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To synthesize and characterize novel ester, amide and ether (from deacetyl) derivatives of xylopic acid.
Methodology: Base catalysed ester formation was employed in the synthesis of the esters while direct coupling with HBTU was employed in the synthesis of the amide derivative. Deacetylation of xylopic acid was achieved by refluxing the acid with 10% methanolic KOH. The structure of xylopic acid and its derivatives were determined using ^{1}H NMR spectroscopy, mass spectrometry and IR spectroscopy.
Results: Xylopic acid was isolated from the dried fruits of *Xylopia aethiopica* at a yield of 0.13%$^{w}/_{w}$. The various derivatives of xylopic acid, namely esters, amide and deacetylated analogues were synthesized in moderate to high yields (47.11- 93.52%) and characterized.
Conclusion: Xylopic acid was isolated from the dried fruits of *Xylopia aethiopica,* crystallized and characterized. The isolated crystals were used to synthesize novel derivatives of xylopic acid and these were also characterized.

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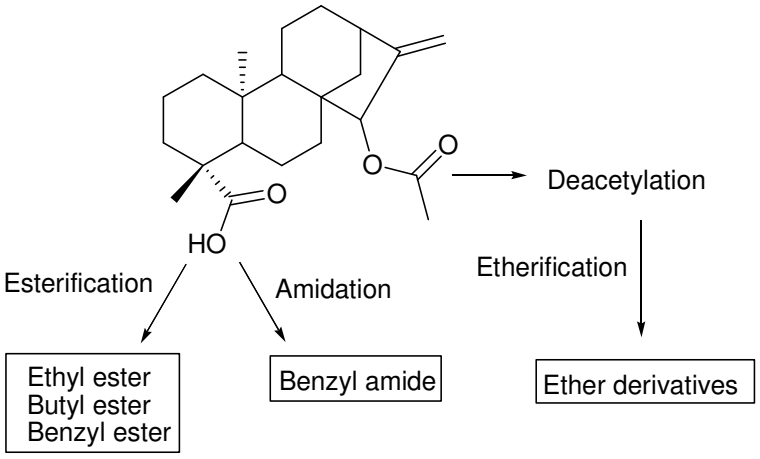
Keywords: Xylopic acid; synthesis; Xylopia aethiopica; esters; amides; deacetyl xylopic acid.

1. INTRODUCTION

Xylopia aethiopica or Ethiopian pepper, as it is usually called, is an angiosperm belonging to the family Annonaceae, and it is among the species that thrive in the evergreen rain forests of tropical and subtropical Africa. It matures as a slim, tall tree of approximately 60 cm in diameter and up to 30 m high, usually has a straight stem, and has a slightly stripped bark. It bears odoriferous fruits, in the form of slender pods slightly curved with about 15 carpels and are arranged in capitula to form bouquets of 12-20 bacciferous-like capsules [1]. *Xylopia* is compressed from the Greek words "*xylon pikron"* which mean "bitter wood". The second part of the plant's binomial name, *aethiopica*, refers to its origin, Ethiopia; however, currently it grows most prominently as a crop in Ghana [2]. The plant has several local names. In Ghana it is known as *'hwenteaa'* in the Akan dialect, *'etso'* and *'so*' in Ewe and Ga dialects respectively, and it is known by the Waala people from the Upper West Region of Ghana as '*samaamdabile'*. This plant has played a key role in African traditional medicine for several centuries owing to its wide array therapeutic indications [3]. *Xylopia aethiopica* is used in the treatment of cough, bronchitis, rheumatism, malaria, uterine fibroid, amenorrhea [4], boils, sores and cuts among others [5].

Xylopic acid, a kaurene diterpene is among the key constituents in the fruits of *Xylopia aethiopica*. Various *in vitro* studies conducted on this compound have revealed that it possesses a wide array of biological and pharmacological properties (e.g. antimicrobial, anticancer, analgesic and anti-inflammatory). Over the years, this compound has been on the cutting edge of various studies conducted in numerous scientific research areas, yielding ground-breaking findings. For instance, in a recent study, Biney et al. [6] investigated various neuropharmacological effects of xylopic acid in an *in vivo* experiment involving mice. Woode et al. [7] also showed that xylopic acid possessed very good analgesic activity. In an earlier study, Boakye-Yiadom et al. [8] had shown that xylopic acid possesses antimicrobial activity against gram positive and gram negative bacteria, and fungi. Elsewhere, Davino et al. [9] also reported that xylopic acid and its epimer, acetylgrandifloric acid exhibited significant antimicrobial activity against test microorganisms, with minimum inhibitory concentration (MIC) greater than or equal to 250 µg/mL. In spite of all these interesting findings, determining the biological activities of xylopic acid derivatives and comparing such activities with that of the isolated acid have received very little attention. Work by Soh et al. [10] reporting that epoxide derivatives of xylopic acid possess good trypanocidal activity against *Trypanosoma brucei* with no detected cytotoxicity, and 15-oxo-ent-kaur-16-en-19-oic acid, obtained by oxidation of xylopic acid exhibited significant cytotoxic effects on MRC-5 fibroblast suggesting its potential to be used as an anticancer agent are few reported biological activities of xylopic acid derivatives. It is against this background that we intend to form novel semi-synthetic derivatives of xylopic acid (scheme 1) that may later be investigated for various biological activities.



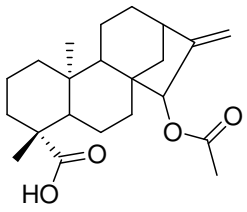
Scheme 1. Synthetic strategies and proposed modifications on xylopic acid

2. EXPERIMENTAL

2.1 General

TLC was carried out using silica gel 60 F254 pre-coated plates and detection was achieved by staining with a saturated solution of ceric sulphate in 10% sulphuric acid followed by heating. Column chromatography was performed using silica gel 60 (70–230 mesh). FTIR spectra were obtained using a Perkin Elmer FTIR spectrum 2 ATR spectrophotometer and "Perkin Elmer Spectrum Version 10.03.09" program. ^{1}H NMR (400 MHz and 500 MHz) spectra were recorded on a "Bruker 400" and "Bruker 500" spectrometers using TMS as an internal standard in DMSO. Data is reported for each compound as follows: chemical shift in ppm (δ), integration, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br s = broad singlet), coupling constant (*J*) was measured in Hertz (Hz), chemical group bearing specified protons. Mass Spectra were obtained using "Waters-Micromass ZQ2000" instrument (LCMS). Melting point determinations were performed with a Stuart digital, SMP10 melting point apparatus and were uncorrected. A magnetic stirrer with heating and ceramic heating plate model "IKA C-MAG HS 7" was used to provide the source of heat for the synthesis. Reagents and solvents used are of commercial grade and were used as supplied without any further purification, except when specified in the experimental procedure.

2.2 Isolation of Xylopic Acid



Dried fruits of *Xylopia aethiopica* were obtained from the Kejetia market in Kumasi, Ghana and authenticated by the Department of Pharmacognosy, KNUST, with a voucher number of FP/09/76. The dried fruits (1.025 kg) were powdered and extracted with 2 litres of petroleum ether (40/60) for 72 hours using cold maceration. The extract was concentrated with a rotavapor (Buchi R 210) at 60℃. 500 mL of ethyl acetate was added to the concentrated mass and allowed to stand for 48 hours to allow formation of xylopic acid crystals. The concentrate was decanted after the period to separate the deposited crystals from the upper oily mass.

The crude xylopic acid crystals (2.130 g) were recrystallized from freshly distilled ethanol and the crystals obtained washed with cold pet ether 40/60 and dried to give xylopic acid as white crystals, 1.318 g (yield 0.13%$^{w}/_{w}$).

Rf (retardation factor): Pet: EtOAc, 9:1 (0.51)

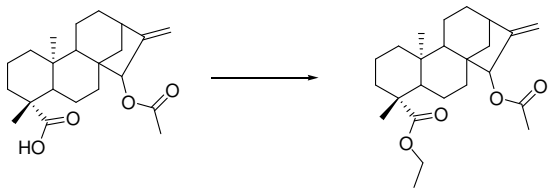
Mp, 264-265 ˚C Lit, 265-266 ℃ [11]

$V_{max}cm^{-1}$; 3280.5, 2927.21, 1722.99, 1704.89, 1271.13, 807.17

δ_H, (400 MHz, DMSO) 12.10 (1H, br s, COO**H**), 5.08 (1H, s, C=C-**H**), 4.97 (1H, s, C**H**), 4.86 (1H, s, C=C-**H**), 2.67 (1H, s, C**H**), 2.17 (3H, s, C$\mathbf{H}_3$), 2.12 -1.17 (9H, m, C**H**, C$\mathbf{H}_2$), 1.16 (3H, s, C$\mathbf{H}_3$), 1.15-0.98 (6H, m, C**H**, C$\mathbf{H}_2$), 0.97 (3H, s, C$\mathbf{H}_3$) 0.96-0.80 (3H, m, C**H**,C$\mathbf{H}_2$).

m/z (ES) 359.1 (M-H, 100%), 360.1 (M-H+1, 24%), 378.1 (M+ H_2O), 379.1 (M+H_2O+1), 301.0 (M -$C_2H_3O_2$)

2.3 Ethyl Ester



A mixture of xylopic acid (0.336 g, 0.93207 mmol) and sodium carbonate (0.300 g, 2.8302 mmol) were placed in a reaction vessel and DMSO (5 mL) added. The mixture was stirred at room temperature for 30 minutes. Ethyl iodide (0.5 mL, 6.2512 mmol) was then added and the mixture was heated at 80℃ while stirring (with monitoring by TLC) for 4 hours. The resulting mixture (which appeared as a reddish brown solution) was then cooled to room temperature and 1 M sodium thiosulphate solution (20 mL) added in a separating funnel, before extracting with ethyl acetate (4×10 mL). The combined organic fraction was dried with anhydrous Na_2SO_4, filtered and evaporated to dryness to give the crude sample which was purified by chromatography (silica gel) [eluent – Pet: Et_2O- 100:0- 30:70] to yield the product (89.04%), as off-white crystals.

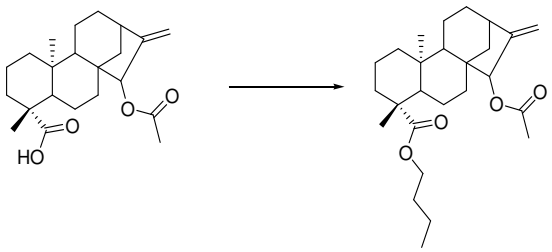
Rf: Pet: Et_2O, 2:1 (0.75)

Mp, 134-135℃

$V_{max}cm^{-1}$; 2923.82, 2854.55, 1737.48, 1712.78, 1456.32, 1225.37, 889.78

δ_H, (400 MHz, DMSO); 5.08 (1H, s, C=C-**H**), 4.90 (1H, s, C**H**), 4.80 (1H, s, C=C-**H**), 4.05 (2H, q, *J*=7.5Hz, C$\mathbf{H}_2$) 2.647 (1H, s, C**H**), 2.35-2.14 (3H, m, C**H**, C$\mathbf{H}_2$), 2.130 (3H, s, C$\mathbf{H}_3$), 2.10-1.3 (9H, m, C**H**, C$\mathbf{H}_2$), 1.20 (3H, t *J*=7.5Hz, C$\mathbf{H}_3$), 1.14 (3H, s, C$\mathbf{H}_3$), 1.00-0.86 (6H, m, C**H**, C$\mathbf{H}_2$), 0.85 (3H, s, C$\mathbf{H}_3$).

2.4 Butyl Ester



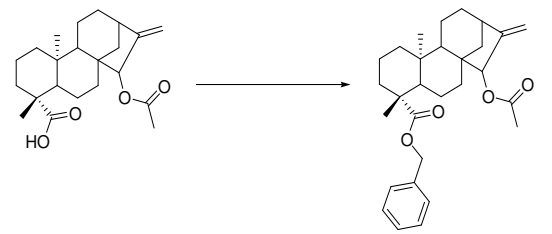
The method described above was used in the synthesis of the butyl ester product (93.37%) which was obtained as a pale yellow gum.

Rf: Pet: Et_2O, 2:1 (0.77)

V_{max}cm-1; 2934.01, 2873.24, 1739.81, 1720.15, 1663.27, 1229.54, 888.23.

δ_H, (400 MHz, DMSO); 5.08 (1H, s, C=C-**H**), 4.98 (1H, s, C**H**), 4.86 (1H, s, C=C-**H**), 4.04 (2H, q *J*=7.0Hz, C$\mathbf{H}_2$), 2.69 (1H, s, C**H**), 2.17 (3H, s, C$\mathbf{H}_3$), 2.12-1.05, (23H, m, C**H**, C$\mathbf{H}_2$),1.16 (3H, s, C$\mathbf{H}_3$), 0.95 (3H, t *J*=7.0Hz, C$\mathbf{H}_3$), 0.87 (3H, s, C$\mathbf{H}_3$).

2.5 Benzyl Ester



Xylopic acid (0.347 g, 0.9626 mmol) and sodium carbonate (0.279 g, 2.8396 mmol) were placed in a reaction vessel and DMSO (5 mL) was added. The mixture was stirred at room temperature for 30 minutes. Benzyl chloride (0.8 mL, 6.9521 mmol) and potassium iodide (0.896 g, 5.3976 mmol) were then added and the mixture stirred at 80℃ (with monitoring by TLC) for 6 hours. The resulting mixture (which occurred as a reddish brown solution) was then cooled to room temperature and the content transferred into a separating funnel. 1 M sodium thiosulphate solution (25 mL) was added before extracting with ethyl acetate (5×10 mL). The combined organic fraction was dried (anhydrous Na_2SO_4), filtered and evaporated to dryness to give the crude sample which was purified by chromatography (silica gel) [eluent – Pet: Et_2O- 100:0- 30:70] to yield the product (51.73%), as a white crystalline solid.

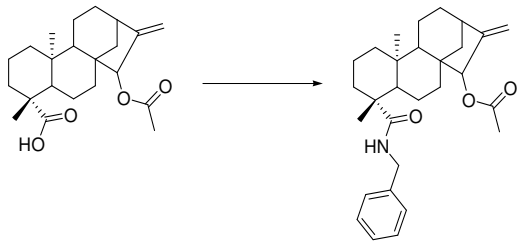
Rf: Pet: Et_2O, 2:1 (0.81)

Mp, 114-115℃

$V_{max}cm^{-1}$; 2926.94, 2865.01, 1738.74, 1723.99, 1441.49, 1367.1, 1225.04, 1040.14, 702.17

δ_H, (400 MHz, DMSO); 7.46-7.37 (5H, m, Ar**H**), 5.06 -5.17 (2H, m, C$\mathbf{H}_2$), 4.97 (1H, s, C=C-**H**), 4.96 (1H, s, C**H**), 4.85 (1H, s, C=C-**H**), 2.67 (1H, s, C**H**), 2.17 (3H, s, C$\mathbf{H}_3$), 2.14-1.34 (12H, m, C**H**, C$\mathbf{H}_2$), 1.19 (3H, s, C$\mathbf{H}_3$), 1.17-0.86 (6H, m, C**H**, C$\mathbf{H}_2$), 0.80 (3H, s, C$\mathbf{H}_3$).

2.6 Benzyl Amide



Xylopic acid (0.100 g, 0.2774 mmol), triethylamine (0.1 mL, 0.7170 mmol) and [N,N,N′,N′-Tetramethyl-O-(1H-benzotriazol-1-yl) uronium hexafluorophosphate] (HBTU) (0.210 g, 0.5537 mmol) were placed in a reaction vessel and DCM (2 mL) was added. The mixture was stirred at room temperature for 1.5 h. Benzyl amine (0.15 mL, 1.3732 mmol) was then added and the mixture stirred continuously (monitoring with TLC) for 18 h. The mixture was concentrated, dissolved in ethyl acetate (5×10 mL) and transferred into a separating funnel. The solution in the separating funnel was washed with distilled water (5×10 mL) followed by brine

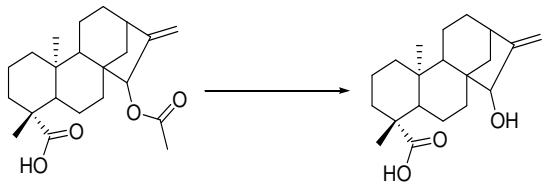
(5×10 mL). The organic fraction was dried with anhydrous Na_2SO_4, filtered and evaporated to give the crude sample which was purified by chromatography (silica gel) [eluent – Pet: Et_2O- 100:0- 30:70] to yield the product, as yellowish gum which solidified upon standing, (47.11%).

Rf: Pet: Et_2O, 2:1 (0.68)

V_{max}cm-1; 2923.72, 2853.36, 1725.62, 1645.55, 1457.74, 961.93, 746.62, 696.75.

δ_H, (500 MHz, DMSO); 7.80-7.20 (5H, m, Ar**H**), 5.03 (1H, s, C=C-**H**), 4.92 (1H, s, C=C-**H**), 4.81 (1H, s, C**H**), 4.77 (1H, s, N**H**), 4.24 -3.94 (2H, m, C**H**2), 2.64 (1H, s, C**H**), 2.14-1.34 (12H, m, C**H**, C**H**2), 2.12 (3H, s, C**H**3), 1.24 (3H, s, C**H**3), 1.11 (3H, s, C**H**3) 1.17-0.86 (6H, m, C**H**, C**H**2),

2.7 Deacetyl Xylopic Acid



A mixture of xylopic acid (0.400 g, 1.1096 mmol) and 10%$^{w}/_{v}$ methanolic KOH (5 mL, 8.9118 mmol) were placed in a reaction vessel and refluxed while stirring continuously (monitoring with TLC) for 2h. The resulting mixture was transferred into a separating funnel. 10% $^{v}/_{v}$ sulphuric acid solution (35 mL) was added and a blue litmus paper was used to monitor the pH to ensure that it is sufficiently acidified. The cloudy solution in the separating funnel was then extracted with ethyl acetate (5×10 mL). The combined organic fraction was dried with anhydrous Na_2SO_4, filtered and evaporated to dryness to give the crude sample which was purified by chromatography (silica gel) [eluent – Pet: Et_2O- 100:0- 30:70] to yield the product, as white crystalline solid, (93.52%).

Rf: Pet: Et_2O, 2:1 (0.29)

Mp, 204-206 ˚C Lit, 204-206 ℃ [12]

V_{max}cm-1; 3391.59, 2939.22, 2856.05, 1684.09, 1469.29, 1444.6, 1396.26, 1226.5, 1174

δ_H, (400 MHz, DMSO); 12.0 (1H, br s, COO**H**) 5.10 (1H, s, C=C-**H**), 5.01 (1H, s, C=C-**H**), 4.81 (1H, s, O**H**), 2.60 (1H, s, C**H**), 2.42-1.21 (12H, m, C**H**, CH_2),1.16 (3H, s, CH_3), 0.99 (3H, s, CH_3) 0.98-0.80 (7H, m, C**H**, CH_2)

m/z (ES) 317.1 (M-H, 100%)$^-$, 318.1 (M-H+1, 22%)$^-$, 336.0 (M+ H_2O)$^+$

3. RESULTS AND DISCUSSION

3.1 Isolation and Characterization of Xylopic Acid

Xylopic acid crystals at a yield of 0.13%$^{w}/_{w}$ were obtained from the isolation procedure. This can be considered as a relatively improved yield when compared with a yield of 0.05%$^{w}/_{w}$ reported by Adosraku and Oppong Kyekyeku [13] who used a similar method for the extraction and isolation of the crystals. Considering an earlier work by Ekong and Ogan, [12] xylopic acid was isolated at a yield of 1.30% which is relatively higher than what was obtained in this experiment. In all these experiments, petroleum ether (40/60) was used in the extraction of xylopic acid, however, Ekong and Ogan used ethyl acetate to recrystallize the extracted xylopic acid. This method of recrystallization was attempted in our investigations with the hope of increasing the yield of xylopic acid, but we observed that the acid dissolved poorly even in relatively large volumes of ethyl acetate and at temperatures as high as 75℃. Hence, ethyl acetate could not be used for recrystallization of xylopic acid in our case. Again, the possibility of using an alternative solvent for extracting the plant material with the hope of increasing the yield of xylopic acid was explored. Chloroform was considered, as a lower volume is required to extract relatively large quantities of xylopic acid even at room temperature. It was later observed that a solution of xylopic acid in chloroform maintained one spot when refrigerated, however, on standing at room temperature for 24 h, multiple spots could be seen on TLC. This observation suggests that xylopic acid may not be stable in chloroform, causing degradation at room temperature. Although the possibility of employing chloroform as solvent in extracting xylopic acid in relatively high yields at low temperatures could be investigated in the future, that route was not pursued in our current studies.

The xylopic acid crystals obtained were first identified by melting point; the crystals had a melting point of 264-265℃. This is comparable with the results obtain by Fahim et al. [11] who determined the melting point of isolated xylopic acid as 265 – 266℃. This is also in close agreement with figures reported by Ekong and Ogan, and Adosraku and Oppong Kyekyeku who determined the melting point of the crystals as

259-260℃ and 260-261℃ respectively. Other tools for identification came from spectral analysis. From the infrared spectrum of the xylopic acid obtained, some major absorption bands could be identified in the functional group region. For instance, the absorption band at 3280.5 cm^{-1} signifies the presence of a hydroxyl (OH) group, whilst a relatively broad band occurring at 3100-2700 cm^{-1} is generally suggestive of a carboxylic acid (-COOH) functional group. Strong absorption bands at 1722.99 and 1704.89 cm^{-1} suggest the presence of two carbonyl functional groups. The peak at 1722.99 cm^{-1} is likely due to the vibration of the carbonyl (C=O) present in the acetyl group, while that at 1704.89 cm^{-1} may be due to carbonyl (C=O) in the carboxylic acid, following the general agreement that carbonyls in esters vibrate at a higher frequency compared with those in carboxylic acids [14]. It is important to indicate that the infrared spectrum obtained in this experiment compares very much with that obtain by Adosraku and Oppong Kyekyeku.

With the mass spectrometric analysis, the ionization method employed in this experiment was the electrospray ionization (ESI). It was observed from our analysis that the xylopic acid ionizes better in the negative mode than in the positive mode, as the signal intensity for the negative ionization (MS ES- :359, 4.4×10^7) is about a thousand times greater than that of the positive ionization (MS ES+:361, 3.3×10^4). In the negative mode, a very intense peak is observed at mass to charge ratio (m/z) of 359.1. This peak corresponds to an $[M–H]^-$ peak, as the predicted molecular weight of the compound is approximately 360, hence loss of a proton will lead to a fragment with a molar mass of approximately 359.

Again, a peak with m/z of 360.1 and having an intensity of approximately 24% could also be observed. This peak is probably not the molecular ion peak but rather may be due to the presence of elements with only two naturally occurring stable isotopes (e.g. H,C and N), and the mass of the more abundant isotope is one mass unit less than the other [(X+1) elements]. Here, the peak at 359.1 represents the X peak while that at 360.1 represents the (X+1) peak. The (X+1) peak in this case is most likely to be due to the presence of ^{13}C rather than ^{2}H because the relative abundance of ^{13}C in nature is 1.09%. Therefore the probability that the $[M-H]^-$ ion contains a ^{13}C isotope [(1.09/98.91)×100 ×22 carbon atoms] is 24.24. This figure mostly corresponds to the intensity of the peak on the mass spectrum [15,16].

Considering the mass spectrum for ionization in the positive mode, an $[M+H]^+$ peak with m/z 361 was not observed as expected, however, peaks at 378.1 and 379.1 are observed instead. The peak at 378.1 may be due to $[M+H_2O]^+$ ion whilst that at m/z of 379.1 represent an (X+1) peak of the latter. There is also another peak at m/z of 301.0, which could represent loss of an acetate group $[M-OCOCH_3]^+$.

From the NMR spectrum of xylopic acid, it was encouraging to observe all the indicative signals that could be attributed to the compound. This includes a broad singlet at chemical shift of 12.05 ppm signifying the presence of a carboxylic acid proton. Again, there are three singlet peaks at 5.08, 4.98 and 4.86, representing 3 protons (3H). Signal at 4.98 ppm corresponds to the C-15 proton (Fig. 1) while the two other peaks represent the two diastereotopic olefinic protons exhibiting geminal anisochrony. This may arise from the fact that one of the olefinic protons interacts more with the deshielding acetyl functional group than the other as a result of the rigidity of the C=C double bond. The proton on C-7 is assigned the signal at chemical shift 2.69 ppm due to its closeness to the strongly deshielding carboxylic acid functional group. The three intense singlets at 2.17, 1.16 and 0.92 ppm probably correspond to the methyl protons on C-27, C-20 and C-19 respectively. This assignment stems from the fact that the protons on C-19 are the least deshielded whilst the protons on C-27 are most deshielded due to their close proximity to the deshielding oxygen atoms.

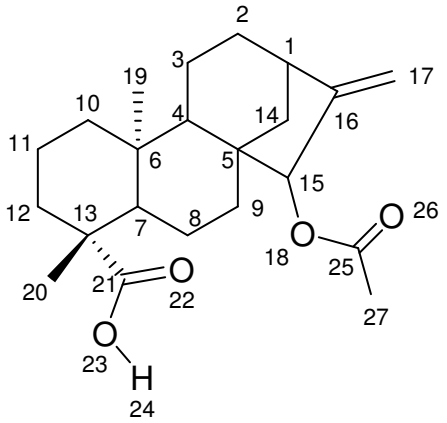


Fig. 1. Structure of xylopic acid showing atom numberings

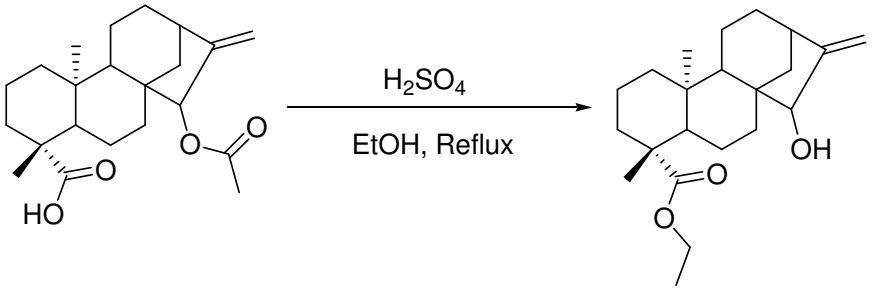
3.2 Synthesis and Characterization of Novel Derivatives

Acid mediated ester formation (Fischer esterification) was first attempted in this experiment, but it appeared that the concentrated sulphuric acid (catalyst) probably interacts undesirably with the xylopic acid in ethanol upon adding the former. An attempt to form an ethyl ester of xylopic acid *via* acid catalysis yielded a product, which appeared to be more polar than the xylopic acid starting material, as evidenced by TLC analysis. It was therefore assumed that there could be a possible cleavage of the acetyl group of xylopic acid upon addition of the sulphuric acid followed by the esterification of the deacetylated xylopic acid, leading to the formation of an ester of deacetyl xylopic acid (Scheme 2). This method of ester formation was therefore abandoned.

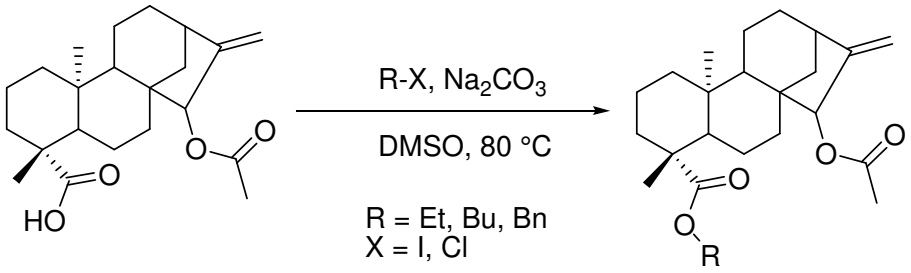
An infrared spectrum of this compound shows no peak at 3280 cm^{-1}, an evidence of the loss of the hydroxyl (OH) group in the xylopic acid. The proton NMR also showed absence of carboxylic acid proton. From the ^1H NMR spectrum obtained for the compound, a 2H quartet is observed at 4.10 ppm and a 3H triplet is observed at 1.15 ppm, which is indicative of a methyl group and a methylene group coupling to each other, both with coupling constant of 7.5 Hz (ethyl group). This will represent the ethyl group of the ethyl ester. A singlet at 3.15 ppm, with integration of 1H, is probably due to the OH group in the deacetylated compound, giving added evidence to our proposed structure (Scheme 2).

The challenges encountered in the acid mediated synthesis prompted us to investigate the esterification under basic conditions. It is gratifying to report that spectral evidence points to successful synthetic outcome. Using the corresponding alkyl iodide and sodium carbonate in DMSO at 80°C gave the desired ester product, (Scheme 3).

From the ^1H NMR spectrum of all the esters and amid it could be observed that the broad singlet at chemical shift of 12.05 ppm attributed to the acid was no longer present, an indication that a transformation involving the loss of the hydroxyl group of the xylopic acid had taken place. In the case of the ethyl ester, a quartet at 4.07-4.01 (J=7.5Hz) with an integration of two protons (2H) and a triplet at 1.20 ppm (J=7.5Hz) which was also integrated as three protons (3H) could be observed in the NMR spectra. This is indicative of ethyl ester formation, and all other signals observed were comparable to that of xylopic acid.



Scheme 2. Proposed reaction for acid catalysed ethyl ester formation



Scheme 3. Base mediated esterification of xylopic acid

Infrared spectrum was also obtained which provided additional information in terms of the structural features of the synthesized compound. The disappearance of an OH stretch at 3280 cm^{-1} and the absence of a broad peak between 2700-3100 cm^{-1} suggest that the carboxylic acid group originally present in xylopic acid has been converted to another functional group, typically an ester. The evidential loss of hydroxyl group of the xylopic acid was observed in all the ester and amide derivatives that were synthesized. The melting point of the ethyl ester derivative which was determined as 134-135℃ does not provide any crucial information as (to the best of our knowledge) there is no such data on the compound. This, however, may be valuable information that could be relied upon as a standard for future investigations.

The progress of most esterification reactions relies on chemical equilibrium, therefore, in these esterification experiments, a relatively large quantity of alkyl halide (excess reagent) was reacted with a comparatively low quantity of xylopic acid (limiting reagent). This ensures that the equilibrium position of the chemical reaction shift towards the formation of the desired product (ester) in high yields. Also, after several trials, the optimum temperature required to favour the desired product formation in a good yield within the shortest time was determined and employed for each of the synthesis experiments. Although the product was isolated in good yield, (89%), we believe this could be further optimised.

Spectral evidence of the structure of the butyl ester was also obtained. Most of the signals observed in the spectrum of the ethyl ester were also observed with some additional signals. For instance, multiplet at 4.07-3.99 ppm, integrating for two protons (2H), is likely to be due to the methylene protons from the alkyl portion of synthesized butyl ester that is directed bonded to the carboxylate group. The triplet at 0.96 ppm integrated as three protons (3H) is also suspected to be due to the methyl protons on the butyl chain of the formed ester.

The infrared spectrum shows the presence of two carbonyl stretch vibrations at 1739.81 and 1720.15 cm^{-1}, indicating the presence of two carbonyl functional groups in the compound (i.e. two ester functional groups).

Due to the appearance of the compound (gum), melting point could not be determined. It is noteworthy that the compound was obtained in very high yield of 93.37%

Furthermore, the benzyl ester, which was obtained as a white crystalline solid, was also taken through various spectroscopic analyses i.e. ^{1}H NMR and IR. Like the other compounds, most of the very meaningful spectral information was obtained from the NMR data.

From the NMR spectrum, there is a multiplet at 7.46-7.37 ppm with an integration of five protons (5H), indicating a mono substituted benzene ring (14). This is in agreement with the proposed structure of the benzyl ester. There is also a multiplet at 5.17-5.06 ppm, which may be due to the methylene protons on the benzyl group. Their apparent multiplet nature may be due to long range coupling to other protons in the compound. All other proton signals appear to be typical of what would be expected of xylopic acid moiety.

The IR spectrum for this compound was comparable to that of the other ester derivatives particularly in the functional group region. However, the fingerprint region showed absorption bands that occur in a unique pattern.

This compound which melts at 114-115℃ was obtained at a relatively low yield of 51.73%. This yield may be improved through, for example, the direct use of a more reactive alkyl halide (i.e. an alkyl iodide) rather than converting a less reactive benzyl halide through the Finkelstein reaction to form the alkyl iodide *in situ*.

The presence of an iodide in one form or another as a reagent in all the synthetic procedures led to the formation of molecular iodine (characterized by reddish brown colouration of reaction system) at the end of the synthesis. This molecule, which appeared to share similar solubility properties with the synthesized esters made separation by column chromatography problematic. However, addition of the thiosulphate solution rapidly reacts with and converts the molecular iodine to water soluble iodide ions which are easily washed off.

The yield of the benzyl amide (of xylopic acid) was rather low, (47.11%). This may probably be due to ineffective activation of xylopic acid by the coupling agent (HBTU) to the active carboxylic acid for subsequent reaction with the amine to form the desired amide.

To determine the structure of the synthesized compound, infrared and ^{1}H NMR spectra were

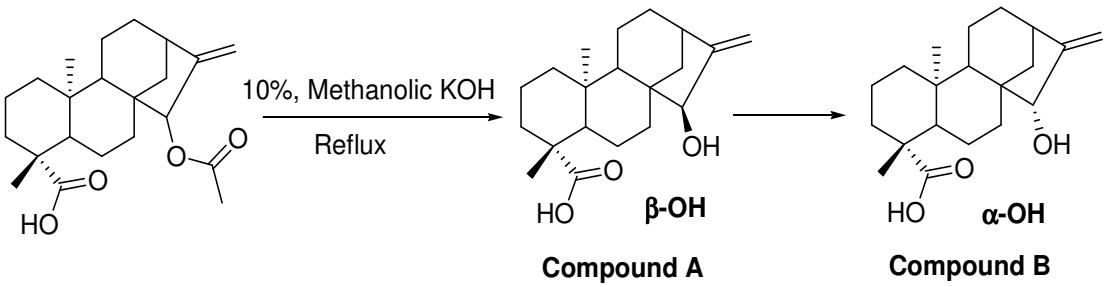
obtained. From the ^{1}H NMR spectrum obtained for the compound, a multiplet is observed at 7.8-7.2 ppm with integration of five protons (5H) is attributed to the monosubstituted benzene ring. The presence of another singlet at 4.77 ppm that has integration of 1H is most probably due to the NH proton in the amide functional group. Finally, the multiplet at 4.24-3.94 ppm (2H) is probably due to the CH_2 protons in the benzyl group thus providing evidence that the structure is a benzyl amide as proposed. Most of the other parts of the spectrum are in agreement with that of xylopic acid.

Again, considering the IR spectrum obtained, the presence of a weak absorption near 3470 cm^{-1} on the spectrum could be attributed to the N-H stretch as these vibrations are usually of low to medium intensity. Again, in the fingerprint region, the presence of a monosubstituted benzene ring is usually characterized by a strong absorption near 690 cm^{-1} and another near 750cm^{-1} [14]. From the spectrum obtained, the occurrence of two strong absorption bands at 696.75 and 746.62 cm^{-1} suggest the probability of the presence of a monosubstituted aromatic system, which in this case would be the benzyl group of the amide synthesized.

Base catalysed ester hydrolysis was employed in the deacetylation of xylopic acid. It was observed that when the reaction was allowed to proceed for 4hrs or over, TLC monitoring showed two compounds which are very close in terms of polarity (Rf values). However, when the reaction was allowed to proceed for 2hrs or below, only one spot was observed. The formation of epimers (α-OH and β-OH) was suspected in the case where two spots were observed on TLC. A solution of potassium hydroxide (KOH) has been shown to cause epimerization, as demonstrated by Mitsuhashi et al, who have shown that treatment of a C/D cis-20-ketosteroid with various concentrations of KOH solution results in epimerization (mixture of 17α- and 17β-H-20-keto compounds), [17].

The two compounds formed from the deacetylation (scheme 4) were successfully separated by column chromatography in the ratio of 25:1 for the compound showing the higher (compound A, β-OH epimer) and lower (compound B, α-OH epimer) Rf values respectively. It can be suggested that deacetylation of xylopic acid initially produces the β-OH epimer, which upon prolonged heating slowly converts to the α-OH epimer, (Scheme 4).

The melting point of compound A which occurred in a larger quantity was determined as 204-206℃. Ekong and Ogan obtained the same values (204-206℃) for a compound identified as (-) kaur-16-en-15β-hydroxy-19-oic acid (i.e. the β-OH epimer of deacetyl xylopic acid) confirming the identity of the compound formed in this experiment [12]. Due to the very low yield of compound B, its melting point could not be determined. However, both compounds were taken through mass spectrometric analysis. The two compounds appeared to be better ionized in the negative mode than in the positive mode. This is because the major functional group in the compound i.e. the carboxylic acid functional group will preferentially be deprotonated rather than being protonated. In the negative mode, a very intense peak is observed at mass to charge ratio (m/z) of 317.1 for both compounds. This peak corresponds to an $[M–H]^-$ peak, as the predicted molecular weight of the compound is 318. Here too, the presence of an (X+1) peak is observed at m/z of 318.1 for both compounds with a peak intensity of about 22%. Due to its very low yield, no further analysis were carried out on compound B, but the mass spectrometric data gives strong indication that it is likely to be an epimer of deacetyl xylopic acid.



Scheme 4. Epimerization of deacetyl xylopic acid

For compound A, positive ionization is very similar to that of xylopic acid in that there is a peak at m/z of 336.0 which may be due to an $[M+H_2O]^+$ ion and another peak at 302.0 possibly due to a loss of a hydroxyl group from the molecular ion $[M-OH]^+$.

The NMR spectrum of compound A shows a broad singlet at chemical shift of 12.0 (1H) suggesting the presence of a carboxylic acid functional group. Again, like xylopic acid, there are three peaks at 5.10, 5.01 and 4.88 with integration of 3 protons (3H). Nevertheless, quite differently, it could be suggested that the signal at chemical shift 4.88 ppm may correspond to the hydroxyl proton (25) while the two other peaks represent the two diastereotopic olefinic protons (i.e. protons on C-17) which exhibit geminal anisochrony (Fig. 2). Again, the proton on C-15 is represented by the signal at chemical shift 3.60 ppm rather than 4.98 ppm, as observed in xylopic acid. The difference could be due to the loss of the acetyl group which consequently results in the loss of one oxygen atom which would otherwise further increase the deshielding (electron withdrawing effect) of the proton on C-15. The occurrence of two intense singlets at 1.16 and 0.92 are due to the methyl protons on C-20 and C-19 respectively. This assignment stems from the fact that the protons on C-19 are deshielded to a lesser extent whilst the protons on C-20 are deshielded much more extensively due to their close proximity with the deshielding oxygen atoms of the carboxylic acid functional group. Interestingly, the singlet due to C-27 occurring at 2.17 in the spectrum for xylopic acid has disappeared. This therefore points to the fact that hydrolysis of xylopic acid has led to a loss of the acetyl group bearing the methyl protons on C-27.

Finally, from the infrared spectrum of the compound, an absorption band at 3391.59 cm^{-1} is indicative of a carboxylic acid O-H stretch while one strong absorption band at 1684.09 cm^{-1} suggests the presence of only one carbonyl functional group in the compound.

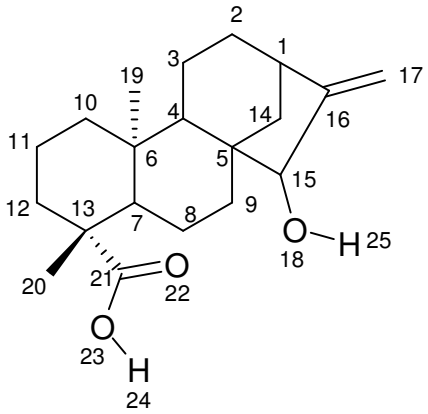
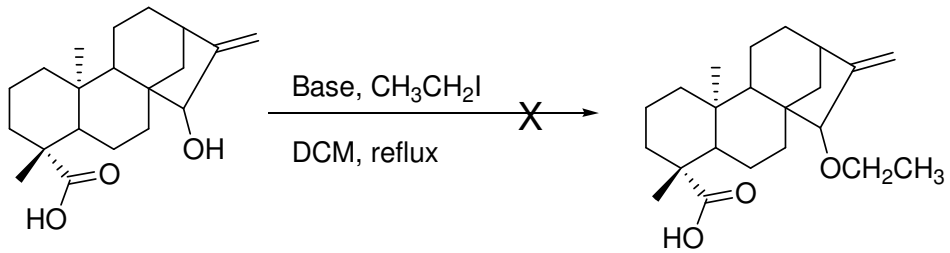


Fig. 2. Structure of deacetyl xylopic acid showing atom numberings

The deacetyl xylopic acid which was previously reported in literature [10,12], was used as starting material in an attempt to synthesize ether derivatives of deacetyl xylopic acid (Scheme 5). The Williamson method of ether synthesis was employed but this proved unsuccessful after several attempts and method variation. It was suspected that the alkoxide ion intermediate could not be formed to allow subsequent reaction with an alkyl halide to produce the desired compound (ether).

This could be because the alkali or base used (apart from sodium metal, KOH, NaOH and Na_2CO_3 were also employed) could not sufficiently ionize the aliphatic alcohol as the latter is generally of low reactivity mainly because it forms part of a very bulky compound. Other bases such as NaH could be investigated for its ability to ionize the 15-OH group in deacetyl xylopic acid to form an alkoxide ion which will be further reacted with an alkyl halide to produce the desired ether derivative.



Scheme 5. Attempted route for ether formation

4. CONCLUSION

Xylopic acid was isolated from the dried fruits of *Xylopia aethiopica,* purified and characterized using mass spectrometry, IR and ^{1}H NMR spectroscopy. The isolated crystals were used to synthesize a range of novel ester and amide derivatives of xylopic acid in moderate to good yields. We have shown in our findings that base mediated process is the most appropriate route for esterification of xylopic acid, as strong acids do cause deacetylation leading to the formation of an undesirable product. Our investigations have also revealed that prolonged heating during base catalysed deacetylation of xylopic acid causes epimerisation and thus any attempts at deacetylation should proceed within short periods. Synthesis of ether derivatives of deacetyl xylopic acid, however would require further investigation. All the synthesized derivatives were also characterized using spectroscopic analysis.

These synthesized derivatives, like many plant isolates, may potentially possess very essential pharmacological or biological activities that may be similar to the biological activities of xylopic acid, or possess improved pharmacological or biological properties. This potential may thus be confirmed through further investigation.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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