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SEMISYNTHESIS OF A MELLEIN-TYPE 3,4-DIHYDROISOCOUMARIN FROM CASHEW NUT SHELL LIQUID (CNSL)

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ABSTRACT

*Mellein-type 3,4-dihydroisocoumarin **39** (i.e., 8-methoxy-3-tridecyl-3,4-dihydroisocoumarin or 8-methoxy-3-tridecylisochroman-1-one), and its precursors, namely, methyl 2-methoxy-6-pentadecylbenzoate (**37**), methyl 2-methoxy-6-pentadecanoylbenzoate (**38**) and (E)-methyl 2-methoxy-6-(pentadec-1-enyl)benzoate (**19**) were synthesized from anacardic acid (**12**) as a starting material obtained from Cashew Nut Shell Liquid (CNSL) in an overall yield of 78%. The transformation of **12** to **39** involved protection of the reactive phenolic and carboxylic acid groups of compound **12** through methylation followed by hydrogenation so as to saturate the mono-, di- and tri-unsaturated C_{15} chains of anacardic acid (**12**). Subsequent benzylic oxidation and reduction of the keto functional group with concomitant dehydration of the alcohol led to the formation of **19**,which after deprotection of* the carboxyl group followed by lactonization, gave the mellein-type 3,4-dihydroisocoumarin **39**.

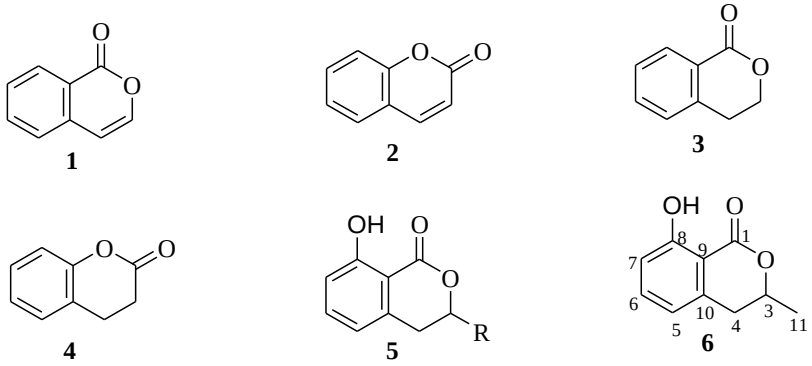
Keywords: Semisynthesis, 3,4-dihydroisoumarin, Anacardic acid, Cashew Nut Shell Liquid (CNSL)

INTRODUCTION

Melleins constitute a family of naturally occurring compounds which consist of a phenol fused to a six membered cyclic ester (lactone) as their fundamental structural feature (Fig. 1). The basic structural motif of the melleins is isomeric to that of the coumarins. Accordingly, the melleins are fittingly called *iso*coumarins to signify that they are, indeed, structural derivatives of *iso*coumarin (**1**).

Melleins are found in natural sources mainly in fungi from genera such as *Aspergillus*, *Ceratocystis*, *Cladosporium*, *Fusarium* and *Penicilium*. They are of limited occurrence in other natural sources such as bacteria, lichens, liverworts, higher plants, insects and marine sponges. Like other isocoumarins, melleins are biosynthetically polyketides (el Khoury and Atoui 2010, Chacón-Morales et al. 2013). The general name mellein is derived from *Aspergillusmelleus*, the fungus from which the well-known (*R*)-(-)-8-hydroxy-3-methyl-3,4-dihydroisocoumarin (**7**) (Fig. 2) was originally isolated (Chacón-Morales et al 2013).

Melleins and related compounds are known to exhibit a wide range of biological and pharmacological activities such as phytotoxic, neurotoxic, antibacterial, antifungal, antimalarial, antiallergic, antitumor, anti-inflammatory, antiulcer, pheromonal and antileukemic.(Chacón-Morales et al. 2013, Kern and Bestmann 1994). Other melleins are known inhibitors of Hepatitis C Virus (HCV) protease (Feng et al. 2010, Sun et al. 2012).



1 = *iso*coumarin or 2H-1-benzopyran-2-one

2 = coumarin or 2*H*-chromen-2-one

3 = 3,4-dihydro*iso*coumarin or *iso*chroman-1-one

4 = 3,4-dihydrocoumarin or chroman-2-one

5 = General structure of melleins or 3,4-dihydro*iso*coumarins, where R = alkyl or aryl

6 = Mellein or 8-hydroxy-3,4-dihydro-3-methyl*iso*coumarin

Figure 1: Structural relationships of *iso*coumarin derivatives (melleins) and coumarins

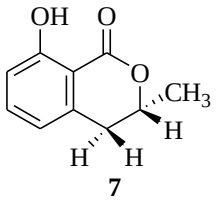


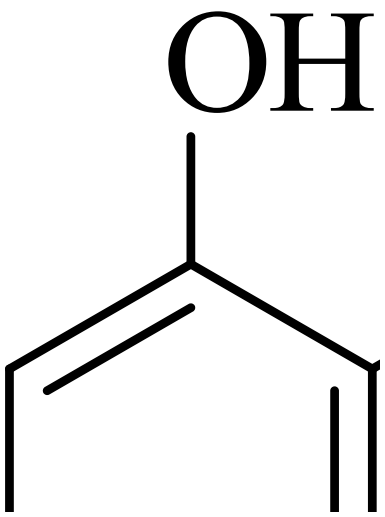
Figure 2: (*R*)-(-)-8-hydroxy-3-methyl-3,4-dihydroisocoumarin

In view of the aforementioned bioactivities and associated potential applications of melleins, a lot of efforts have been directed at studying these compounds. As part of the ongoing efforts to add value to the cashew crop by preparing useful products utilizing Cashew Nut Shell Liquid (CNSL) from the agro-waste Cashew Nut Shells (CNS), a semisynthetic approach was envisioned towards some melleins from the anacardic acid component of CNSL. Thus, the aim of this work was to transform anacardic acid (**12**) (Fig. 3) through a series of reactions to some melleins such as **7**-**10** and other structurally related 3,4-dihydroisocoumarins *via* the aldehyde **18** (Scheme 1) as the key intermediate(Kadir 2017). The basis of the planned approach is the retrosynthetic analysis for the targeted melleins, which is summarized in Scheme 1.

Cashew Nut Shell Liquid

CNSL is a dark brown viscous natural oil obtained as a by-product in the cashew nut processing industries.It consists mainly of four phenolic compounds, the proportion of which depends on the method by which it is obtained from the shells. Figure 4 shows the four phenolic constituents of CNSL namely: anacardic acid (**12**), cardanol (**20**), cardol (**21**), and methylcardol (**22**) (Omanakuttan et al. 2012, Mdachi 2013, Mkungu et al. 2013, Hamad and Mubofu 2015).

Figure 3: Anacardic acid (**12**) as a mixture of the saturated, monoene, diene and triene side chain



Scheme 1: Retrosynthetic analysis of melleins**7** to **10**

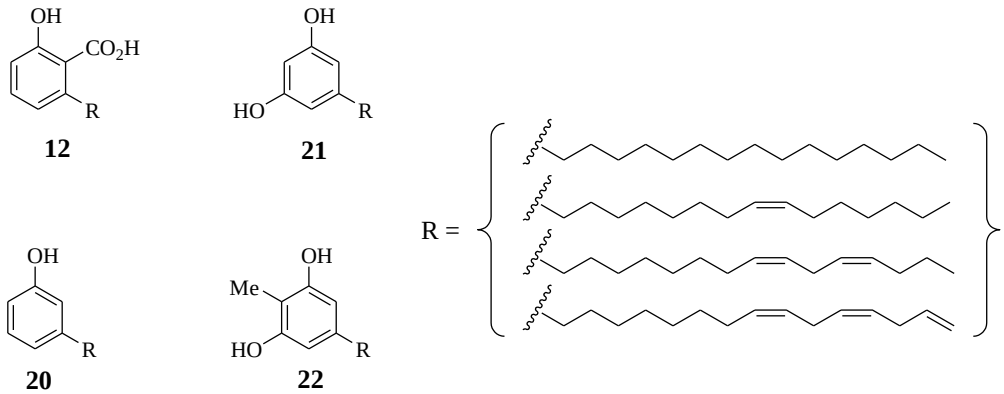


Figure 4: The major phenolic constituents of CNSL

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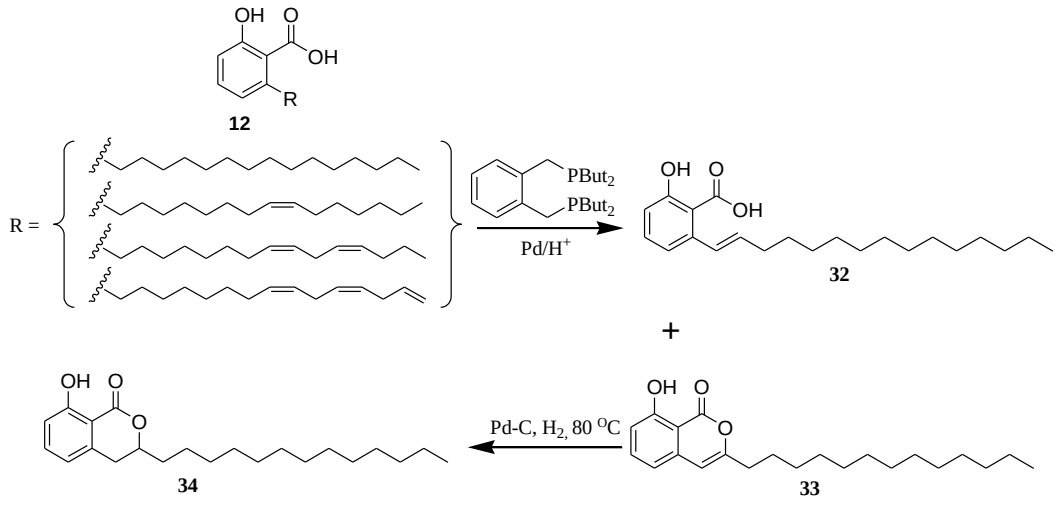
A variety of applications of CNSL hadbeen reported (Mdachi 2013, Edogaet al. 2006, Pimentel et al. 2009).Isolated constituent phenols from CNSL, especially cardanol and anacardic acid,hadbeen extensively utilized as starting materials in the syntheses of useful chemical products (Mdachi 2013, Mkungu et al. 2013). As an input to the worldwide efforts and interest in the exploitation of bio-raw materials in the syntheses of valuable chemical products, we proposed a synthetic strategy that would make use of anacardic acid to synthesize the naturally occurring bioactive mulleins **7**-**10**.

Melleins (3,4-dihydroisocoumarins)

Theenantiomeric melleins**7** and **8** (Scheme 1)are the structural parents of the mellein family and themselves occur in both stereochemical forms (Donner et al. 2004). Along with their 4-hydroxylated congeners **9** and **10** (Scheme 1), the melleins are known for their diverse bioactivities including, among others, antibacterial, antimalarial, antifungal and anticancer activities (Chacón-Morales et al. 2013, Hazalin et al. 2013, Ióca et al. 2014, Santiago et al. 2014).

Mgayaet. al.(2015) reported on the synthesis of crystalline unsaturated lactone, 8-hydroxy-3-tridecyl-1H-isochrome-1-one (**33**) by isomerization of anacardic acid (**12**), followed by hydrogenation of compound (**33**) to produce a saturated lactone, 8-hydroxy-3-tridecyl-3,4-dihydroisochromen-1-one (**34**). Isomerization of monoeneanacardic acid (**12**) resulted in a crystalline isoanacardic acid, (*E*)-2-hydroxy-6-(pentadec-1-enyl)benzoic acid (**32**) as a major product. This was then metathesized with 2-butene to give 3-prop-1-enylphenol (**33**). Both isomerization reactions used a 1,2-*bis*(ditertiarybutylphosphinenomethyl)benzene modified palladium catalyst as shown in scheme 2 below.



Scheme 2: Synthesis of unsaturated (**33**), saturated (**34**) benzolactones and isomerized anacardic acid (**32**).

Recently, a conversion of anacardic acid (**12**) to compound **19** was carried out (Kisula et al. 2015). This compound is one of the key intermediates towards the planned synthesis of the melleins**7**-**10** as well as the

mellein-type 3,4-dihydroisocoumarin **39** that is described in thispaper.

MATERIALS AND METHOD

Materials, Reagents, Instruments and General Procedures

Cashew nut shells (CNS) were collected as industrial waste from Cashew Nuts Ltd in Dar es Salaam.They were soaked in petroleum ether so as to extract Cashew Nut Shell Liquid (CNSL) from which anacardic acid was isolated and subjected to a series of reactions. All reagents and chemicals used in this study were purchased from Sigma Aldrich, South-Africa and used as received. All glassware apparatus used were cleaned and oven-dried before use. Organic layers obtained following work up of reaction mixtures were dried over magnesium sulphate ($MgSO_4$). Column chromatographic separations were performed using EM type 755500 MFC silica gel (60-120 mesh).Thin Layer Chromatography (TLC) analysis was performed on Merck pre-coated silica gel ($60F_{254}$/0.2 mm) plates and spots were visualized under UV light. The structures of compounds were elucidated by using FTIR, H-NMR and ^{13}C-NMR at the University of Dar es Salaam, Department of Chemistry in Tanzania and University of Witwatersrand, South Africa. FT-IR spectrometer Bruker Optic GmbH 2011 was used for IR data acquisition. ^{1}H-NMR and ^{13}C NMR spectra were recorded in deuterated chloroform ($CDCl_3$) at 300 and 100 MHz respectively on Bruker A.G. spectrometer with tetramethyl silane as the internal standard and chemical shifts (δ) are reported in parts per million (ppm). Coupling constants are reported as *J* (Hz) and signal multiplicities are abbreviated as: doublet (d), doublet of doublet (dd), triplet (t), quartet (q), broad (br) and multiplet (m).

Extraction of Cashew Nut Shell Liquid

Cashew Nut Shells (1000 g) were soaked in petroleum ether (2500 mL) and left aside for four days (2 × 2500 mL). The dark brownish solution obtained was decanted to give 5000 mL of CNSL extract. The solvent was removed under reduced pressure using a rotary evaporator at 60 °C to give 122.00 g(12.2% yield) as a brownish oil which was used without further purification.

Isolation of Anacardic Acid

The method used by Dholakiyaet al.(2012)forthe isolation of anacardic acid from CNSL was adapted with modification. The CNSL extract (62.00 g) was dissolved in 5% aqueous methanol (400 mL) followed by addition of calcium hydroxide (31.00 g) in portions while stirring. After complete addition, the temperature of the reaction mixture was raised to 50 °C and stirred for 3 hours to form a calcium anacardate precipitate which was then filtered, washed with methanol (50 mL), and dried in an oven for 2 hours to obtain compound **35** as brown solid (88.00 g).Compound **35** (55.00 g) was suspended in 6 M HCl (220 mL) and stirred for 1 hour at room temperature. The reaction mixture was extracted with ethyl acetate (2 × 75 mL) and the combined organic layer was washed with distilledwater (3 × 50 mL), dried over anhydrous magnesium sulphate and decanted. The organic layer obtained was concentrated under reduced pressure to give anacardic acid (**12**) (45.00 g, 97.21 % yield) which was used as a starting material. FT-IR(film): 3450-2500 cm^{-1} (br), 3008.91 cm^{-1}(w), 2924.35 cm^{-1} (s), 2853.81 cm^{-1} (s), 1710.42 cm^{-1} (m), 1662.29 cm^{-1}(m), 1606.01 cm^{-1} (m), 1576.58 cm^{-1} (m), 1449.92 cm^{-1} (m).

2-hydroxy-6-pentadecylbenzoic acid (36)

Compound **12** (20.00 g, 0.06 mol) was dissolved in methanol (40 mL) and 10% palladium-on-carbon (0.25 g) catalyst was added (Palasova and Cervery 1999). The mixture was then autoclaved while bubbling in hydrogen gas at room temperature for 10 hrs, after which it was filtered using a celite bed to remove the catalyst. The filtrate was concentrated under reduced pressure to

obtain 2-hydroxy-6-pentadecyl-benzoic acid (19.60 g, 0.056 mol, 93%) as a gray solid.FT-IR (film): 2955.83 cm^{-1} (w), 2915.32 cm^{-1} (s), 2849.16 cm^{-1} (s), 1704.72 cm^{-1} (s), 651.73 cm^{-1} (s), 1603.90 cm^{-1} (m), 1445.29 cm^{-1} (s)

Methyl 2-methoxy-6-pentadecylbenzoate (37)

Compound **36** (2.00 g, 5.74 mmol) was methylated using a literature method (Shieh etal. 2002, Bernini et al. 2011) to afford 1.80 g (83.27%) of desired compound as pale yellow solid. FT-IR (film): 2921.36 cm^{-1} (s), 2851.94 cm^{-1} (s), 1739.49 cm^{-1} (s), 1713.59 cm^{-1} (m), 1659.81 cm^{-1} (m), 1603.03 cm^{-1} (m). ^{1}H NMR(300 MHz, $CDCl_3$):δ_H= 0.88 (t, 3H, *J* = 6 Hz), 1.23 (m, 20H), 1.57 (m, 2H), 2.65 (t, 2H, *J* = 6 Hz), 3.72 (s, 3H), 3 74 (s, 3H), 6.98 (d, 1H, *J* = 8 Hz), 7.12 (d, 1H,*J* = 8 Hz), 7.49 (t, 1H, *J* = 8 Hz)

Methyl 2-methoxy-6-pentadecanoylbenzoate (38)

Compound **37** (1.00 g, 2.65 mmol) dissolved in dichloromethane (30 mL) was placed in a flask followed by addition of potassium permanganate (3.00 g, 18.9 mmol) and active manganese dioxide (0.40 g, 4.6 mmol) (Shaabani et al. 2004). [Active manganese dioxide was freshly prepared by adapting a literature procedure (Carpina 1970)].The reaction mixture was stirred for 48 hours at room temperature. After 8 hours of stirring, 5 drops of sulfuric acid were added and the mixture was allowed to continue stirring for further 40hrs. The progress of the reaction was monitored by TLC. After the reaction was complete, the mixture was filtered and the residue was extracted with dichloromethane (2 × 10 mL), dried over $MgSO_4$, and concentrated to give compound **38** (0.89 g, 85.7.%) as a yellowish liquid. FT-IR(film): 2921.73 cm^{-1} (s), 2852.50 cm^{-1} (s), 1738.52 cm^{-1} (s), 1710.34 cm^{-1} (s), 1600.12 cm^{-1} (m), 1585.08 cm^{-1} (w), 1465.18 cm^{-1} (m), 1376.38 cm^{-1}(m). ^{1}H NMR(300 MHz, $CDCl_3$):δ_H= 0.88 (t, 3H, J = 6 Hz), 1.23 (m, 21H), 1.55 (m, 2H), 2.62 (q, 2H,J = 6 Hz), 3.78 (s, 3H), 3 78 (s, 3H), 7.23 (d, 1H, J = 8 Hz), 7.36 (t, 1H, J = 8 Hz), 7.95 (d, 1H,J = 8 Hz).

Methyl 2-methoxy-6-pentadec-1-enylbenzoate (19)

To compound **38** (0.70 g, 1.79 mmol) in a three-necked round bottom flask was added wet SiO_2 (0.19 g) and stirred for 5 min followed by addition of fine powder of $NaBH_4$ (0.11 g, 3.23 mmol) (Zeynizadeh and Behyar 2005). The reaction mixture was heated at 80 ^oC for 20 minutes while monitoring progress by TLC. The reactionmixture was cooled to room temperature, extracted using dichloromethane (3×6mL), washed with water (2 × 10 mL) and then dried over anhydrous $MgSO_4$ and filtered. The solvent was then evaporated *in vacuo* to give a crude product, which was purified by column chromatography (silica gel, 90:10, petroleum ether/ethyl ether) to yield compound **19** (0.50 g, 74.8%) as yellowish oil, FT-IR(film): 3025.72 cm^{-1} (s), 2922.62 cm^{-1} (s), 2852.86 cm^{-1} (s), 1737.50 cm^{-1} (s), 1604.00 cm^{-1} (m), 1495.42 cm^{-1} (m). ^{1}H NMR(300 MHz, $CDCl_3$): δ_H = 0.88 (t, 3H, J = 6 Hz), 1.23 (m, 19H), 1.55 (m, 2H), 2.19 (q, 2H, J = 6 Hz), 3.76 (s, 3H), 3.79 (s, 3H), 6.01 (m, 1H), 6.66 (d, 1H), 6.91 (d, 1H,J = 8 Hz 7.46 (d 1H, J = 8 Hz 7.51(t, 1H, J = 8 Hz).

8-Methoxy-3-tridecyl-3,4-dihydroisocoumarin (39)

To a stirred solution of compound **19** (0.40 g, 1.00 mmol) in methylene chloride (25 mL), anhydrous $AlCl_3$ was added and the mixture stirred for 4 hours at room temperature (Mali et al. 1992). The reaction was monitored by TLC. After the reaction was complete the resulting mixture was filtered, then dichloromethane (5 mL) was added to the resultant materialsthen concentrated *in vacuo* to give compound **39** (0.25 g, 65.4% yield). FT-IR(film): 2921.63 cm^{-1} (s), 2852.21cm^{-1} (s), 1738.67cm^{-1} (s),

1599.43 cm^{-1}, 1588.19 cm^{-1} (m), 1466.09 cm^{-1} (m). ^1H NMR (300 MHz, $CDCl_3$): δ_H =0.88 (t, 3H, J= 6 Hz), 1.23 (m, 19H), 1.38 (m, 2H), 1.52 (m, 2H), 2.93 (m, 2H), 3.27 (s, 3H), 5.08 (m, 1H), 6.92 (d, 1H, J = 8 Hz), 7.11 (d, 1H, J = 8 Hz), 7.32 (t, 1H, J = 8 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C = 14.13, 22.72, 29.73, 30.83, 33.51, 35.82, 51.46, 79.73, 109.00, 112.01, 130.24, 141.42, 156.27, 174.47 and 179.14.

Cytotoxic Activity

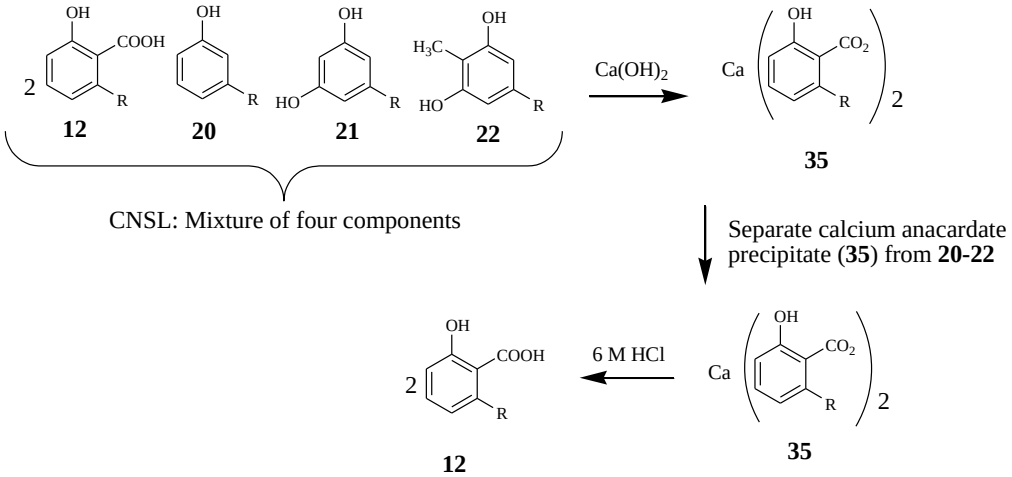
The Brine Shrimp Test (BST) was used to establish the above activity with slightly modification at Muhimbili University of Health and Allied Sciences (MUHAS) Tanzania following standard procedure using brine shrimp (*Artemia Salina* Leach) larvae as indicator organism(Sreeshmal and Nail 2014, Sudhakesavan et al. 2011).

Samples of synthesized compound **39** were prepared in different concentrations (240, 120, 80, 40, 24 and 8 µg/mL) by dissolving 40 mg/mL in dimethyl sulphoxide (DMSO) then adding into vials, each containing 10 brine shrimps larvae and finally adjusting the volume to 5 mL with artificial sea water. The test experiment was done in duplicates. The negative control contained brine shrimp, artificial sea water and DMSO (0.6%) only. The vials containing larvae were incubated under the light for 24 hrs.(Moshi et al. 2010). The dead larvae were counted and recorded while the mean was subjected to analysis using *Fig P* computer program (BiosoftInc, USA). The toxicity of compound **39** was determined from LC_{50} value per dose.

RESULTS AND DISCUSSION

Anacardic Acid (12) from CNSL

Isolation of anacardic acid was achieved by precipitation of CNSL as calcium anacardate salt (**35**) by reacting with calcium hydroxide (31 g) followed by hydrolysis of the salt with HCl to give the free acid as a brown oil (Scheme 3). The infra- red spectrum showed a broad absorption band at 3450-2500 cm^{-1} indicating the presence of O-H stretch for hydroxyl and carboxylic acid groups. A sharp absorption band at 3008.91 cm^{-1} was due to a C-H stretch of sp^2 hybridised carbon, whereas the strong absorption bands at 2924.35 cm^{-1} and 2853.81 cm^{-1} are attributed to a C-H stretch of sp^3 hybridized carbon and the band at 1606.01 cm^{-1} is for the C=C stretch of aromatic ring. The spectroscopic data obtained for anacardic acid was in complete agreement with that reported previously (Kisula et al. 2015).



Scheme 3: Reaction of CNSL to form the anacardic acid

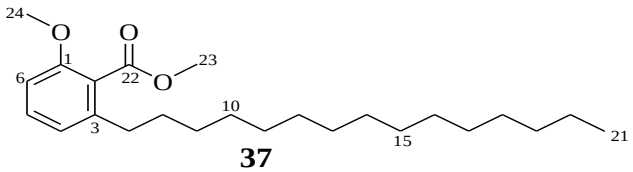
Mellein-type 3,4-dihydroisocoumarin 36
Hydrogenation of **12** gave compound **36** as grey solid (Scheme 4) with the saturated C_{15} side chain. FT-IR spectrumshowed disappearance of the absorption band of sp^2 hybridization at 3008.91 cm^{-1} which meant hydrogenation successfully took place. Strong peaks around 2915.32 cm^{-1} and 2849.16 cm^{-1} indicates the existence of sp^3carbon hybridization and the peak around 1651 cm^{-1} showed the presence of C=C aromatic stretching.

The scheme below summarizes the complete synthetic transformation of anacardic acid (**12**) to 8-methoxy-3-tridecyl-isochroman-1-one (**39**) and other precursors.

Scheme 4: Synthetic Transformation of Anacardic acid (**12**) to the Mellein-type 3,4-dihydroisocoumarin **39** and other precursors

Methyl 2-methoxy-6-pentadecylbenzoate (**37**) was obtained by methylation of compound **36** in 78 % yield as a pale yellow liquid (Shieh et al. 2002, Bernini et al. 2011). The IR spectrum of the compound showed the disappearance of broad bands around 3450 cm^{-1} and 2500 cm^{-1} which indicated a full protection of phenol and acid group and the presence of strong absorption bands at 1739.49 cm^{-1} and 1713.59 cm^{-1} indicated the existence of an ester and ether functional groups, respectively. The ^1H NMR spectrum of compound **37** exhibited signals for aromatic protons at δ_H 6.98, 7.12 and 7.49 ppm as two doublets and a triplet of one proton each for H-6, H-4 and H-5, respectively. Two singlets at δ_H 3.70 and 3.72 indicated the presence of the carbomethoxy and methoxy groups (H-23 and H-24), respectively. A multiplet at chemical shift 2.65 ppm was assigned to the two protons of the benzylic position. A

multiplet of (2H) resonating at chemical shift of 1.57 ppm was assigned to the methylene proton at position number 8 near the benzylic carbon. The twenty protons of the remaining methylene groups appeared at δ_H 1.23 ppm, while the terminal methyl group of the pentadecyl side chain was observed as a triplet of three protons at 0.88 ppm.



37

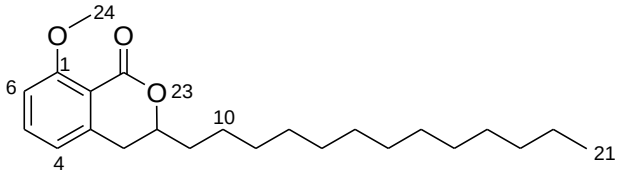
Figure 5: Carbon atomic numbering for compound **37**

Benzylic oxidation of **37** using $KMnO_4$ supported on active manganese dioxide (Shaabani et al. 2004) gave the target keto compound **38** (Scheme 6) as a yellowish liquid. The FTIR spectrum of compound **38**showed a strong and sharp adsorption peak at 1710.34 cm^{-1} indicating C=O stretch of carbonyl functional group which suggested that oxidation of benzyl position took place successfully. However an absorption peak at 1738.52 cm^{-1} indicated the presence of an ester functional group as protected group which suggested that this group was intact and therefore resistant to the oxidation under the conditions applied. Absorptions at 2921.73 cm^{-1} and 2852.44 cm^{-1} indicated saturation along the alkyl side chain group, while the presence of a benzene ring was evident fromthe strong and sharp peak at 1600.12 cm^{-1}. The ^1H NMR spectrum for this compound had its aromatic protons signals appearing at δ_H7.95, 7.23 and 7.36 ppm as two doublets and a triplet for H-4, H-6 and H-5, respectively. Each of these signals represented one proton. It showed two singlets at δ_H 3.76 and 3.78 ppm (6 H) for the two carbomethoxy and methoxy groups (H-24 and H-23, respectively). A multiplet at δ_H2.62 ppm was assignedto the two protons next to the benzylic position (H-8). A multiplet (2H) resonating at 1.55 ppm was assigned to the methylene proton at position 9 while that at δ_H 1.23 ppm was assigned to the twenty two protons of the remaining methylene groups (22H). The terminal methyl group protons (H-21) appeared as a triplet at δ_H 0.88 ppm.

Reductionof compound **38**(Zeynizadeh and Behyar 2005) gave the benzylic alkene **19** as yellow solids. Water in this case plays the role of solubilizing $NaBH_4$ resulting into its fine dispersion on silica gel for substrate interaction. The IR spectrum of the product **19** showed a disappearance of the band at 1710.34 cm^{-1} which indicates the reduction of C=O keto function group and the appearance of a new band at 3025.72 cm^{-1} for sp^2 C-H stretching indicating the formation of C=C of an alkyl chain while the existence of the ester and benzene ring was justified by the peaks around and 1737.50 cm^{-1} and 1604.00 cm^{-1} respectively. The ^1H NMR spectrum of this compoundshowed signals for the aromatic protons at δ_H 6.91, 7.51, and 7.46 ppm as two doublets and one triplet, respectively, each of these signals represented one proton. This data was in complete agreement with that observed by other researchers (Kisula et al. 2015, Godfrey P 2016). The two olefinic protons appeared as multiplets at δ_H6.01 and 6.66, respectively and the two singlets at δ_H3.76 and 3.79 ppm, each representing three protons, were assigned to H-24 and H-23, respectively. A quartet at δ_H 2.19 ppm is due to the two methylene protons at position C-9. A multiplet at δ_H1.55 ppm was assigned to

the two homoallylic methylene protons at C-10 whereas the rest of methylene (CH_2) group protons appeared as a multiplet at δ_H1.23 ppm. The terminal methyl group protons (H-21) of the pentadecenyl side chain were observed at δ_H 0.88 as triplet (3H).

After obtaining compound **19**, efforts were made to convert it into the desired compound **39**, whereby it was treated with anhydrous $AlCl_3$ in methylene chloride at room temperature for 4 hours (Mali et al. 1992). The IR spectrum of compound **39**showed peaks around 2921.63 $cm^{-1}$and 2852.21cm^{-1} representing C-H stretch and the peak at 1738.67cm^{-1}indicating the formation of a lactone ring and the peak around 1585 cm^{-1} represents the presence of a benzene ring. The ^{1}H NMR spectrum of **39**had its aromatic protons appearing at δ_H 6.92, 7.11, and 7.32 ppm as two doublets and one triplet, respectively; each of these signals represented one proton. A multiplet at δ_H5.08 ppm accounts for the proton at CH-O (8H) while a singlet signal for the methoxy group appeared at δ_H 3.82. A multiplet of protons resonating at chemical shift 2.93-1.23 was assigned to the methyl groups at positions 7, 9 and 20, respectively. A triplet at δ_H0.88 ppm wasassigned to the terminal methyl protons of the pentadecyl side chain.The ^{13}C NMR spectrum fully agrees with the assigned structure (Appendix 2.0). In the low field part of the spectrum one resonance appeared at δ_C179.14 ppm which is assigned to a carbonyl carbon of compound **36**. Six signals for the aromatic carbons apeared at δ_C174.47, 156.27 141.42, 130.24, 122.01 and 109.00 ppm while for the methoxy apeared at 51.46 ppm. A signal at δ_C 79.73 ppm was assigned to the carbon attached to an oxygen atom while signal at δ_C14.12 ppm was assigned to the terminal methyl carbon of the pentadecyl chain and the methylene carbon next to it appeared at δ_C22.71 ppm. The rest twelve methylene carbons were shown to appear at chemical shift 29.72 ppm. These $\delta_C$values are comparable to those reported by other researchers (Mgaya et al. 2015). The chemical shifts for benzene carbons appeared at 116.49, 118.37, 136.48 and 139.9 ppm where a slight difference is due to the presence of the different functional group, -OCH_3 in the benzene ring.



39

Figure 6: Carbon atomic numbering for compound **39**

Brine Shrimp Lethality Test Results

Figure 7 shows the plots of the percentage mortality against the logarithms of concentrations of the synthesized compound which gave LC_{50} value 756.9807 µg/ml. This results for the cytotoxicity of compound **39** indicated that the compound **39** is biologically active due to the ability to kill the nauplii whereas the mortality rate were increasing with increasing concentration of the synthesized compound but the compound was not toxic due to its LC_{50} being below 1000 (756.9807 µg/ml) (Hamidi et al. 2014).

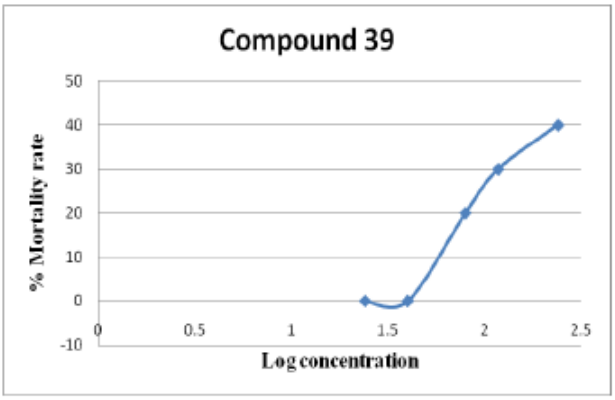


Figure 7: The graph of Mortality rate Vs Log Concentration (µg/ml)

CODE	REGRESSION EQUATIONS	LC_{50} (µg/ml)	REGRESSION COEFFICIENT - R^2
Compound **39**	Y =40.822logx – 67.53	756.9807	0.9326
Cyclophosphamide/standard drug	Y = 69.968logx – 34.93	16.365	0.9949

Figure 8: LC_{50} for standard drug and Compound **39**

CONCLUSIONS

Anacardic acid (**12**), from CNSL which is a cheap and locally available biodegradable renewable agro-waste,was successfully transformed to mellein-type **39**and other precursors. Reduction of the keto functional group and deprotection of methoxy group using $AlCl_3$ led to the target mellein-type compound **39** which has been found to be biologically active and not toxic. Due to smaller amounts of the synthesized compounds available, only BST was managed to be done while the rest of the proposed bioassays were not performed. By utilizing the good and simple protective procedure successfully employed in this work which was previously challenging compounds such as mellein**7-10** can be synthesized.

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