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Triterpenoids from the Bark of *Alstonia Spathulata* Bl. (Apocynaceae)

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Abstract : In Malaysia, *Alstonia spathulata* Bl. is locally known as 'pulai basong' which distribute in peat forest. The separation of the chemical components from hexane crude extract (bark) of *Alstonia spathulata* was carried out using different chromatographic techniques (column chromatography and thin layer chromatography). Two compounds were isolated and identified as lupeol acetate **1** and stigmasterol **2**. These compounds have never been reported and isolated from this species. The structures of these compounds were elucidated based on the basis of detailed 1D-NMR (^{1}H, ^{13}C and DEPT) and 2D-NMR (COSY, HSQC, HMBC) spectroscopic analysis, involving also comparison with data from the literature.

Keywords: Apocynaceae, *Alstonia spathulata*, lupeol acetate, stigmasterol

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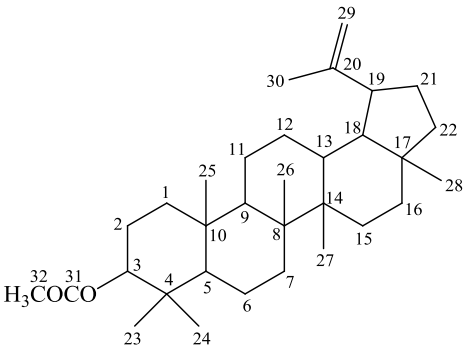

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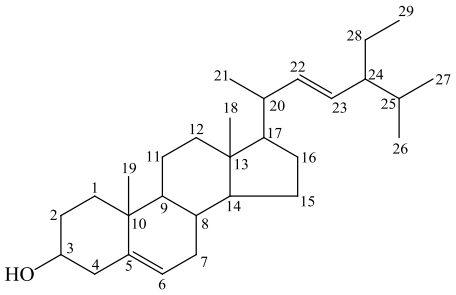

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Introduction

Alstonia is genus that under tribe of Alstonieae in Rauvolfioideae subfamily of Apocynaceae and widely distributed at subtropical Africa, Central America, Southeast Asia, Polynesia and Australia, with most species in the Malesian region [1]. *Alstonia* is the genus of laticiferous shrubs or trees. The plant contain a milky sap which rich in poisonous alkaloids. *A. spathulata* is swamp dweller. The tree is pagoda-like crown. The bark is smooth, grayish and shows abundant of milky latex [2, 3]. In Malaysia, *Alstonia spathulata* Bl. is locally known as 'pulai basong'. In Cambodia, Laos, Vietnam, and, Malaysia, the latex from *Alstonia spathulata* Bl. is applied externally to sores and diseased skin. The bark is used to lower fever and to expel worms from the intestine [3]. The aim of this study is to isolate and identify the chemical compounds from the bark of *Alstonia spathulata.* In this paper, we report the isolation and characterization of lupeol acetate **1** and stigmasterol **2** which are the first time isolated from this species.

Materials and methods

General Methods

Nuclear Magnetic Resonance spectra (NMR) were recorded in deuterated chloroform (CDCl3) on a JEOL 600MHz and 500MHz. Chemical shifts (δ) were reported in ppm and coupling constants (*J*) in Hz. Mass spectra (MS) were determined by using the GC-mass spectroscopy (GC-MS Agilent 5975 Series). The infrared spectra (IR) were recorded on a Nicolet 6700 FTIR spectrophotometer, with CH_2Cl_2 as dilution solvent of the sample. Column chromatography were prepared by using Silica Gel 60F, 70-230 mesh ASTM and, 230-400 mesh ASTM and Silica Gel 60 containing Gypsum F_{254} as stationary phase. Analytical thin layer

chromatography (TLC) was performed on commercially precoated aluminium supported silica gel $60F_{254}$ TLC sheets.

Plant Material

The species selected for the current study is *Alstonia spathulata* Bl. which was collected from Mersing, Johor and identified by the phytochemical group, Chemistry Department, Faculty Science, University of Malaya. The Voucher specimens were deposited at the Chemistry Department, Faculty Science, University of Malaya, Kuala Lumpur, Malaysia and the Herbarium of the Forest Research Institute, Kepong, Malaysia.

Extraction and Isolation

Dried grounded bark of the *Alstonia spathulata* was extracted with hexane for 17 hours at room temperature. The extract was dried by using rotary evaporator. For preliminary fractionation, the hexane crude extract was subjected to column chromatography over silica gel and eluted with different combinations solvent systems of hexane, dichloromethane and methanol mixture by increasing its polarity. Eluents were collected in fractions and were concentrated using rotary-evaporator. Each concentrated fractions was analysed by using aluminium supported TLC plate with a suitable solvent system and test with 5% sulphuric acid. The fractions having spots with same retention factor (R_f) value were grouped together. Each grouped of fractions were treated separately by extensive column chromatography or preparative TLC for further purification process until single spot show on the TLC.

Results and Discussion

Compound **1** was obtained as colourless amorphous. The IR spectrum showed stretching band for ester carbonyl (1712cm^{-1}) and C-O (1263 cm^{-1}) (Figure 1). The mass spectrum of **1** showed a molecular ion peak at m/z 468 $[M]^+$ corresponding to molecular formula $C_{32}H_{52}O_2$. The ^{1}H NMR spectrum (Table 1 and Figure 2) of **1** exhibited six methyl singlets at δ 0.84, 0.83, 0.85, 1.03, 0.94, 0.78 and a vinylic methyl at 1.68 ppm which corresponding to proton attached to C- 23, C-24, C 25, C-26, C-27, C- 28 and C-30 respectively. It was identified as triterpene acetate due to the presence of an acetate methyl signal at δ 2.05 as a singlet. H-3 showed proton signal at δ 4.47 (*dd*, *J* = 10.7 Hz, 5.5 Hz). The presence of two exo-methylene at δ 4.57 and δ 4.69 (H-29) and one methyl signal at δ1.68 (H-30) reminiscent lupane skeleton.

Analysis of ^{13}C NMR spectrum (Table 1 and Figure 3) showed the presence of thirty two carbon atoms indicating a mono acetyl derivative. There are six quaternary carbons δ 37.8 (C-4), δ 40.9 (C-8), δ 37.1 (C-10), δ 42.8 (C-14), δ 43.0 (C-17), δ 151.0 (C-20); six methine carbons δ 81.0 (C-3), δ 55.4 (C-5), δ 50.3 (C-9), δ 38.0 (C-13), δ 48.3 (C-18), δ 48.0 (C-19); eleven methylene carbons δ 38.4 (C-1), δ 23.7 (C-2), δ 18.0 (C-6), δ 34.2 (C-7), δ 20.9 (C-11), δ 25.1 (C-12), δ 27.4 (C-15), δ 35.6 (C-16), δ 29.8 (C-21), δ 40.0 (C-22), δ 109.4 (C-29); eight methyl carbons δ 28.0 (C-23), δ 16.5 (C-24), δ 16.2 (C-25), δ 16.0 (C-26), δ 14.5 (C-27), δ 18.2 (C-28), δ 19.3 (C-30), δ 21.3 (C-32) and the present of a carbonyl group at δ 171.1 (C-31). The alkene carbon appeared at δ 151.0 (C-20) and δ 109.4 (C-29). ^{1}H and ^{13}C NMR spectral data and mass spectrum were in agreement with those reported for lupeol acetate [4, 5]. Thus, compound **1** was identified as lupeol acetate.

Compound **2** was obtained as colourless needles (m.p: 151.0 - 152.0˚C). The mass spectral data of **2** gave a molecular formula $C_{29}H_{42}O$, m/z 413$[M+H]^+$. The IR spectrum displayed stretching band for O-H (3298 cm^{-1}), olefin (1640 cm^{-1}) and C-O (1019 cm^{-1}) (Figure 4). ^{1}H NMR spectrum (Table 2 and Figure 5) consisted of two methyl singlets at δ 0.68 (H-18) and δ 1.00 (H-19), methyl doublets at δ 1.18 (H-21), δ 0.85 (*d*, *J* = 4.1 Hz, H-26) and δ 0.79 (*d*, *J* = 6.8Hz, H-27) and a methyl tripet at δ 0.80 (H-29). A hydroxyl methine proton resonated at δ 3.52 as a multiplet corresponding to proton (H-3). Three olefinic protons in which the last two are of trans configuration were observed at δ 5.35, δ 5.14 (*dd*, *J* = 15.5 Hz and 8.6 Hz) and δ 5.00 (*dd*, *J* = 15.5 Hz and 9.2 Hz) attributable to H-6, H-22 and H-23 respectively.

^{13}C NMR spectrum (Table 2 and Figure 6) of compound **2** exhibited twenty nine signals. Signal for six methyl carbons C-18, C-19, C-21, C-26, C-27 and C-29 could be seen in the most upfield region of the NMR spectrum at δ 12.0, δ 19.5, δ 21.2, δ 21.2 δ 19.1 and δ 12.1. The position of three quaternary carbons appreared at δ 140.8 (C-5), δ 36.6 (C-10), δ 42.3 (C-13); nine methylene carbons: δ 37.3 (C-1), δ 31.7 (C-2), δ 42.4 (C-4), δ 31.9 (C-7), δ 21.2 (C-11), δ 39.8 (C-12), δ 24.4 (C-15), δ 29.0 (C-16), δ 29.8 (C-28); and eleven methine carbons: δ 71.9 (C-3), δ 121.8 (C-6), δ 31.9 (C-8), δ 50.2 (C-9), δ 56.8 (C-14), δ 56.0 (C-17), δ 40.6 (C-20), δ 138.4 (C-22), δ 129.3 (C-23), δ 51.3 (C-24), δ 45.9 (C-25). Signal at δ 140.8, δ 121.8, δ 138.3 and δ 129.3 were observed for four olefinic carbons, corresponding to C-5, C-6, C-22 and C-23 respectively. A deshielded signal of δ 71.9 confirmed the presence of hydroxyl group attached to C-3. This compound was found to be a C-5 unsaturated modified tetracyclic triterpene, which showed only 29 carbons instead of 30 carbons for tetracyclic triterpenes. Comparison of these ^{1}H and ^{13}C NMR data with literature [6, 7] showed that compund **2** is stigmasterol.

Table 1 : ^{1}H NMR [600 MHz, δ_H (*J*, Hz)] and ^{13}C NMR [100 MHz, δ_C] of 1 in $CDCl_3$

Position	δ_H (*J* Hz), ppm	δ_C, ppm	DEPT	Lupeol acetate [75, 125 MHz, CDCl3] [5]
1	0.99 (*m*)	38.4	CH_2	38.6
2	1.62 (*m*)	23.7	CH_2	21.7
3	4.47 (*dd*, 10.7 and 5.5 Hz)	81.0	CH	81.2
4		37.8	Q	38.0
5	0.79 (*m*)	55.4	CH	55.6
6	1.50 (*m*)	18.2	CH_2	18.4
	1.38 (*m*)			
7	1.49 (*m*)	34.2	CH_2	34.4
	1.38 (*m*)			
8		40.9	Q	41.0
9	1.28 (*m*)	50.3	CH	50.5
10		37.1	Q	37.3
11	1.39 (*m*)	20.9	CH_2	21.1
	1.21 (*m*)			
12	1.66 (*m*)	25.1	CH_2	24.0
	1.05 (*m*)			
13	1.65 (*m*)	38.0	CH	36.2
14		42.8	Q	43.0
15	1.67 (*m*)	27.4	CH_2	25.3
	1.00 (*m*)			
16	1.47 (*m*)	35.6	CH_2	35.8
	1.36 (*m*)			
17		43.0	Q	43.2
18	1.35 (*m*)	48.3	CH	48.5
19	2.40 (*m*)	48.0	CH	48.2
20		151.0	Q	151.2
21	1.91 (*m*)	29.8	CH_2	30.0
	1.33 (*m*)			
22	1.38 (*m*)	40.0	CH_2	40.2
	1.18 (*m*)			
23	0.84 (*s*)	28.0	CH_3	27.6
24	0.83 (*s*)	16.5	CH_3	16.7
25	0.85 (*s*)	16.2	CH_3	16.4
26	1.03 (*s*)	16.0	CH_3	16.2
27	0.94 (*s*)	14.5	CH_3	14.7
28	0.78 (*s*)	18.0	CH_3	18.2
29	4.69 (*br, s*)	109.4	CH_2	109.6
	4.57 (*br, s*)			
30	1.68 (*s*)	19.3	CH_3	19.5
31		171.1	C=O	171.3
32	2.05 (*s*)	21.3	CH_3	28.2

Table 2 : ^{1}H NMR [500 MHz, δ_H (*J*, Hz)] and ^{13}C NMR [100 MHz, δ_C] of 2 in $CDCl_3$

Position	δ_H (*J* Hz), ppm	δ_C, ppm	DEPT	Stigmasterol, [100MHz, $CDCl_3$] [2]
1	1.07 (*m*)	37.3	CH_2	37.3
	1.83 (*m*)			
2	1.52 (*m*)	31.7	CH_2	31.6
	1.83 (*m*)			
3	3.52 (*m*)	71.9	CH	71.8
4	2.23 (*m*)	42.4	CH_2	42.3
	2.28 (*dd* 4.6 and 1.7 Hz)			
5		140.8	Q	140.8
6	5.35 (*br, s*)	121.8	CH	121.7
7	1.46 (*m*)	31.9	CH_2	31.9
	1.99 (*m*)			
8	1.52 (*m*)	31.9	CH	31.9
9	0.92 (*m*)	50.2	CH	51.2
10		36.6	Q	36.5
11	1.52 (*m*)	21.2	CH_2	21.1
12	1.15 (*m*)	39.8	CH_2	39.7
	2.02 (*m*)			
13		42.3	Q	42.3
14	0.99 (*m*)	56.8	CH	56.9
15	1.02 (*m*)	24.4	CH_2	24.4
	1.58 (*m*)			
16	1.25(*m*)	29.0	CH_2	28.4
	1.68 (*m*)			
17	1.07 (*m*)	56.0	CH	56.1
18	0.68 (*s*)	12.0	CH_3	11.0
19	1.00 (*s*)	19.5	CH_3	21.2
20	2.02 (*m*)	40.6	CH	40.5
21	1.18 (*d*, 5.7Hz)	21.2	CH_3	21.2
22	5.14 (*dd*, 15.5 and 8.6 Hz)	138.3	CH	138.3
23	5.00 (*dd*, 15.5 and 9.2 Hz)	129.3	CH	129.3
24	1.52 (*m*)	51.3	CH	51.2
25	0.92 (*m*)	45.9	CH	31.9
26	0.85 (*d*, 4.1 Hz)	21.2	CH_3	21.2
27	0.79 (*d*, 6.8Hz)	19.1	CH_3	19.0
28	1.25 (*m*)	29.8	CH_2	25.4
29	0.80 (*t*, 6.9Hz)	12.1	CH_3	12.1

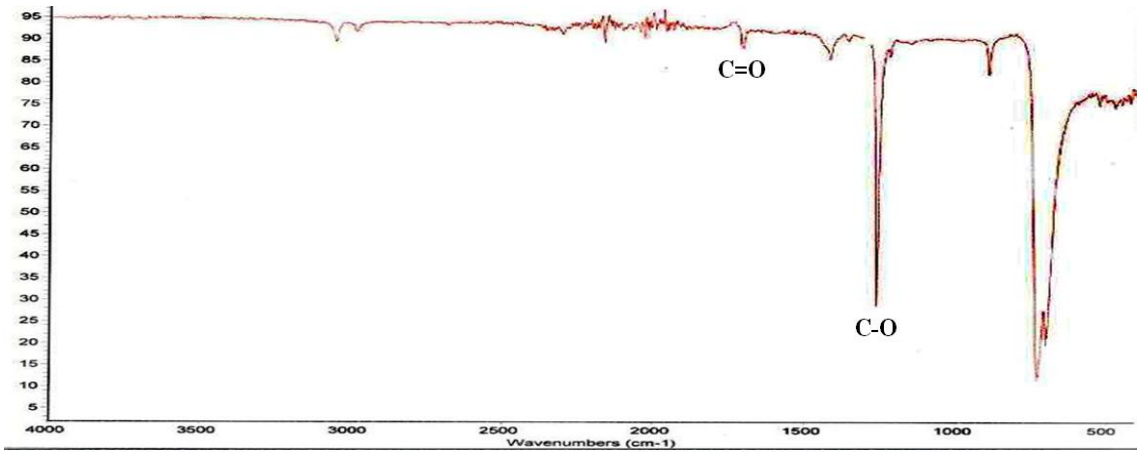

Figure 1 : IR spectrum of compound 1

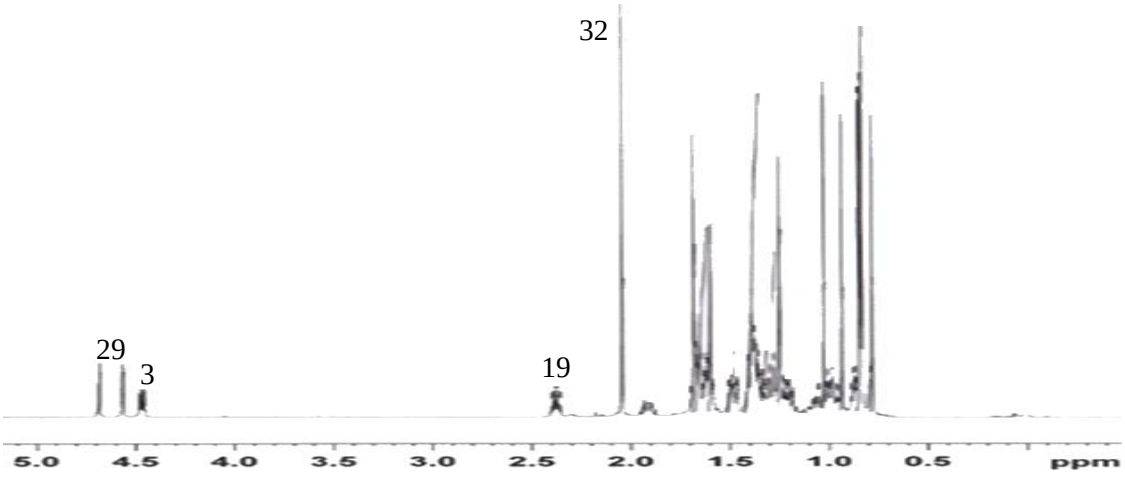

Figure 2 : ^{1}H NMR spectrum of compound 1

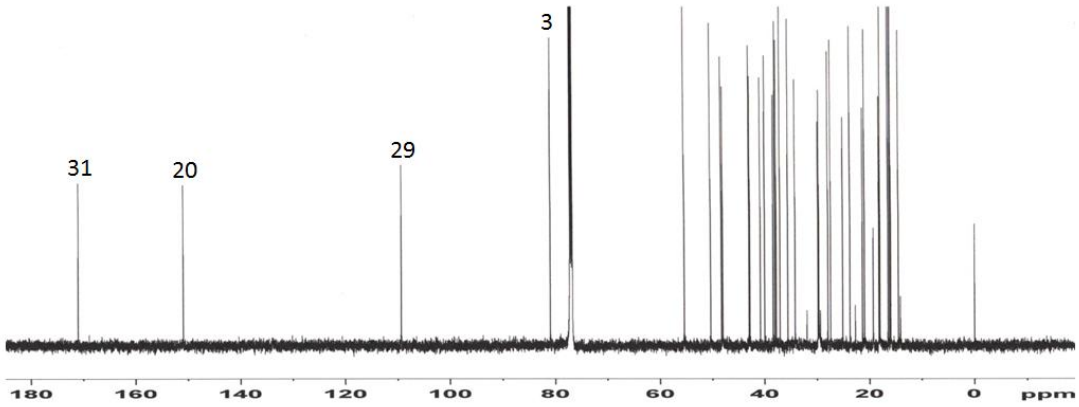

Figure 3 : ^{13}C NMR spectrum of compound 1

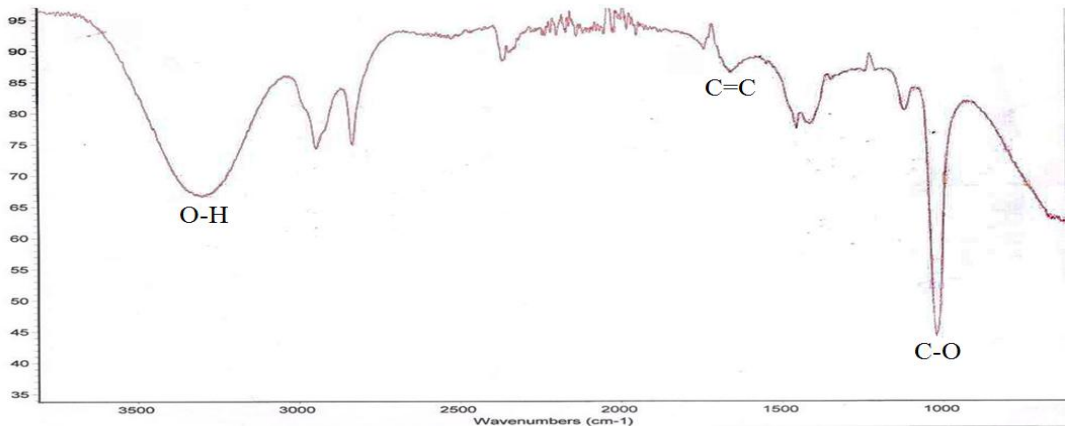

Figure 4 : IR spectrum of compound 2

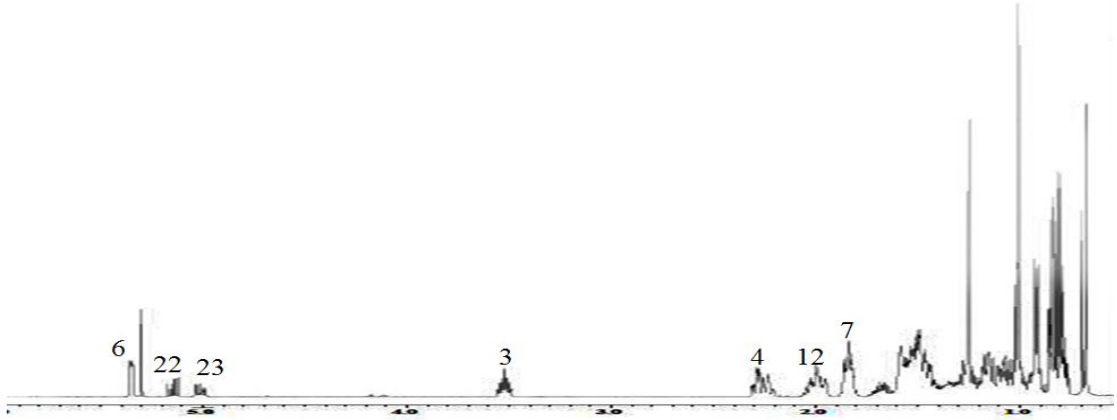

Figure 5 : ^{1}H NMR spectrum of compund 2

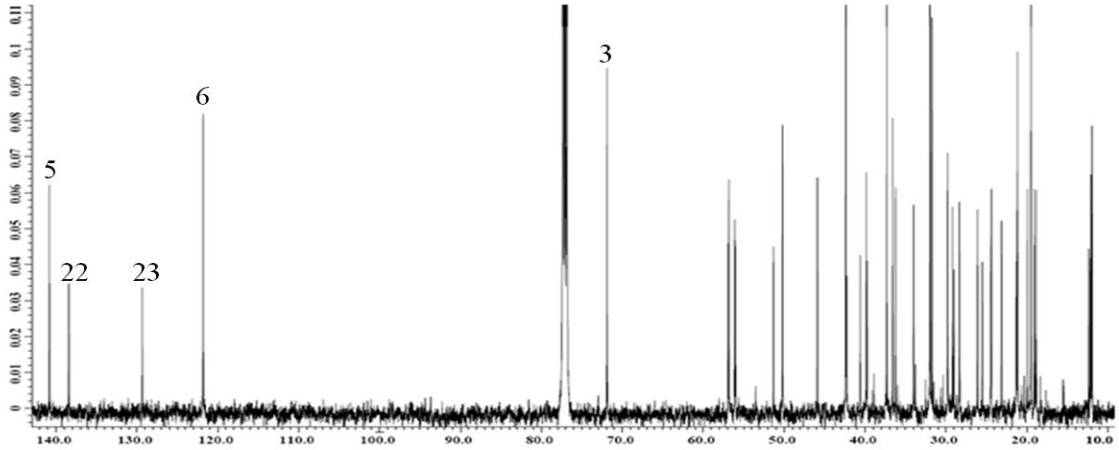

Figure 6 : ^{13}C NMR spectrum of compound 2

Conclusions

The research work through a systematic chemical investigation has determined and identified for the first time the presence of lupeol acetate **1** and stigmasterol **2** from the bark of *Alstonia spathulata.* The work was carried out by means of various physical (solvent extraction and column chromatography) and spectral techniques. The assignment will be further supported by 2D-NMR (COSY, HSQC, and HMBC) spectroscopic analysis. The structures of the compounds were also elucidated by comparison with literature.

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