MANSOURA JOURNAL OF CHEMISTRY
Official Journal of Faculty of Science, Mansoura University, Egypt
E-mail: scimag@mans.edu.eg ISSN: 2974-4938

Phytochemical composition of *thevetia peruviana* leaves

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Received:23/12/2019
Accepted:19/2/2020

Abstract: Phytochemical investigation of Thevetia peruviana leaves has resulted in isolation and identification of 12β-hydroxy-4,16-pregnadien-3,20-dione, using several chromatographic and spectral techniques, beside identification of thirty-seven volatile constituents of a biological interests from the pet. ether and the methylene chloride extracts using GC/MS analysis. The volatile constituents belonging to different classes, acetogenins (fat derivatives), terpenes, shikimates (phenolic constituents) and pregnanes.

keywords: Thevetia peruviana, Apocynaceae, leaves, 12β-hydroxy-4,16-pregnadien-3,20-dione, GC/MS analysis

1.Introduction

Thevetia peruviana, (pers) K Shum, commonly known as yellow oleander, belongs to the family Apocynaceae, a well-known evergreen ornamental shrub, which grows widespread as a wild plant in the tropics and subtropics [1].

Highly concentrated toxic glycosides distributed in the seeds and the roots of the plant, causing digoxin-like effects and targeting the inhibition of the Na^+-K^+ ATPase enzyme in the cardiovascular system [2]. The milky sap of *T. peruviana* contains a heart stimulant compound called Thevetin [3].

The leaves have been reported to contain flavonoids، iridoid glycosides، monoterpenes, triterpenes and cardiac glycosides [4].

T. peruviana scientifically studied for its medicinal value, and showed anti-inflammatory, antimicrobial and used to treat asthma, arthritis, allergies, strong immunomodulatory, strong antioxidant [5,6] and anticancer activities [7], also showed HIV-1 reverse transcriptase, HIV-1 integrase inhibitory activities [3] and used as wound healing potential [8]. *T. peruviana* regarded as potential source of biologically active compounds as anti-termite [3,9], antispermatogenic [3,10], and rodenticide [11].

Herein, this study was directed to identify the bioactive phytochemicals produced from the less polar extracts, pet. ether and methylene chloride, of *Thevetia peruviana* leaves using various chromatographic and spectral methods.

Results and discussion

The chemical analysis of pet. ether and methylene chloride extracts of *T. peruviana* leaves have yielded thirty-eight phytochemicals which were characterized based on GC/MS and NMR analyses.

By means of GC/MS technique, the pet. ether and methylene chloride fractions of *T. peruviana* leaves were analyzed for their volatile constituents (Table 1). Every integrated peak in the GC chromatogram was quantized and compared with EI-MS databases (NIST and WIELY libraries) in order to deduce its chemical identity. A vast variety of volatile compounds were recognized as secondary metabolites which belonging to different classes of naturally occurring compounds such as, acetogenins (fat derivatives), terpenes, shikimates (phenolic constituents) and pregnanes.

About eighteen volatile components, presenting (86.23%) of the whole composition, were identified from pet. ether fraction of *T. peruviana* leaves. The main constituents were methyl linolenate (27.02%) which has anti-virulence potential against *Pseudomonas aeruginosa* and reduces rhamnolipid production by the organisms [12], methyl palmitate (20.08%) which was described to have nematicidal, pesticidal, antibacterial, anti-inflammatory activities [13], antioxidant, hypocholesterolemic [14] and antifungal activities [15] in addition to, methyl linoleate (13.90%) which well-known by its antifungal and antioxidant [16] anti-inflammatory, lipid metabolism regulator, antisecretoric, anthelmintic (Nematodes) and anti-infective activities [13].

Nineteen volatile phytochemicals (69.06%) were matched and identified from the methylene chloride fraction and the most predominant constituents were β-butoxyethanol (13.71%) which used as a solvent in pharmaceutical products and consumer (cosmetics, detergent) applications, also used as a formulation solvent for herbicides, insecticides and as a co-solvent in diesel fuel [17], Loliolide (15.22%) which was used as a natural repellent compound, growth inhibition, germination inhibition and phytotoxic activities for plants, beside its antitumor and antimicrobial activities for animals and microorganisms [18] along with its anti-inflammatory, antiperistaltic and anthelmintic actions [13] and 4-((1E)-3-hydroxy-1-propenyl)-2-methoxyphenol (4.57%) which was found to be used as antioxidant, antimicrobial and anti-inflammatory agent [19

Table (1): Volatile constituents identified from pet. ether and methylene chloride fractions of *Thevetia peruviana* leaves.

Volatile Component Name	R_t	Area%		M.F	m/z (ret. int. %)
		pet.ether	CH_2Cl_2		
2,4,5-Trimethyl-1,3-dioxolane **(1)**	7.69	2.96		$C_6H_{12}O_2$	116 (10) $[M^+]$, 43 (100) $[C_2H_3O]^+$, 44 (67) $[C_2H_4O]^+$, 101 (60) $[C_5H_9O_2]^+$, 73 (43) $[C_3H_5O_2]^+$, 45 (40) $[CHO_2]^+$, 55 (37) $[C_3H_3O]^+$.
β-Butoxyethanol **(2)**	11.47		13.71	$C_6H_{14}O_2$	118 (1) $[M^+]$, 57 (100) $[C_3H_9]^+$, 87 (20) $[C_5H_{11}O]^+$, 45 (43) $[C_2H_5O]^+$.
1-Heptene **(3)**	20.77		2.54	C_7H_{14}	98 (10) $[M^+]$, 41 (100) $[C_3H_5]^+$, 55 (53) $[C_4H_7]^+$, 56 (60) $[C_4H_8]^+$, 43 (40) $[C_3H_7]^+$, 70 (20) $[C_5H_{10}]^+$.
2,6-Dimethylundecane **(4)**	20.89	0.13		$C_{13}H_{28}$	184 (1) $[M^+]$, 57 (100) $[C_4H_9]^+$, 43 (57) $[C_3H_7]^+$, 71 (43) $[C_5H_{11}]^+$, 41 (38) $[C_3H_5]^+$, 55 (23) $[C_4H_7]^+$.
3-Ethyl-4-methyl-1H-pyrrole-2,5-dione **(5)**	21.40		1.02	$C_7H_9NO_2$	139 (100) $[M^+]$, 67 (99) $[C_4H_3O]^+$, 53 (50) $[C_4H_5]^+$, 40 (38) $[C_3H_4]^+$, 41 (37) $[C_3H_5]^+$, 43 (32) $[CHNO]^+$.
(E,E)- 6,10-dimethyl-5,9-dodecadien-2-one **(6)**	24.17		2.03	$C_{14}H_{24}O$	208 (1) $[M^+]$, 43 (100) $[C_2H_3O]^+$, 72 (67) $[C_4H_8O]^+$, 57 (40) $[C_3H_5O]^+$, 59 (30) $[C_3H_7O]^+$, 41 (25) $[C_3H_5]^+$.
1-Methoxy-2-methyl-1-propene **(7)**	24.90		2.03	$C_5H_{10}O$	86 (67) $[M^+]$, 71 (100) $[C_4H_7O]^+$, 43 (50) $[C_3H_7]^+$, 41 (22) $[C_2HO]^+$, 53 (13) $[C_4H_5]^+$.
Dihydroactinidiolide **(8)**	29.90		1.02	$C_{11}H_{16}O_2$	180 (17) $[M^+]$, 111 (100) $[C_8H_{15}]^+$, 43 (77) $[C_3H_7]^+$, 109 (53) $[C_7H_9O]^+$, 67 (43) $[C_5H_7]^+$, 41 (40) $[C_2HO]^+$, 95 (23) $[C_6H_7O]^+$.
1,4-Dimethyl-δ-3-tetrahydroacetophenone **(9)**	32.02		2.54	$C_{10}H_{16}O$	152 (13) $[M^+]$, 109 (100) $[C_8H_{13}]^+$, 43 (93) $[C_2H_3O]^+$, 41 (37) $[C_3H_5]^+$.
Pulegone **(10)**	32.29		3.05	$C_{10}H_{16}O$	152 (10) $[M^+]$, 108 (100) $[C_8H_{12}]^+$, 109 (35) $[C_7H_9O]^+$, 43 (30) $[C_2H_3O]^+$, 91 (18) $[C_7H_7]^+$,41 (18) $[C_3H_5]^+$.
3-(1-Methylhept-1-enyl)-5-methyl-2,5-dihydrofuran-2-one **(11)**	32.84		3.06	$C_{13}H_{20}O_2$	208 (7) $[M^+]$, 43 (100) $[C_3H_7]^+$, 125 (33) $[C_7H_9O_2]^+$, 41 (28) $[C_3H_5]^+$, 107 (23) $[C_7H_7O]^+$, 109 (22) $[C_7H_9O]^+$.
4-(2,2,6-Trimethyl-7-	33.25		3.55	$C_{13}H_{20}O_2$	208 (1) $[M^+]$, 43 (100) $[C_2H_3O]^+$, 123

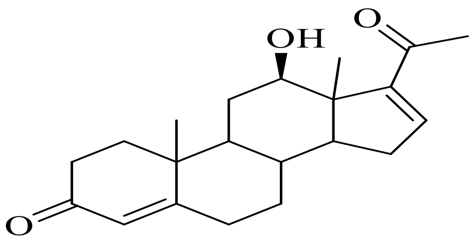
oxabicyclo[4.1.0]hept-1-yl)-3-buten-2-one **(12)**					(99) $[C_8H_{11}O]^+$, 193 (33) $[C_{12}H_{17}O_2]^+$, 41 (23) $[C_3H_5]^+$, 91 (15) $[C_7H_7]^+$.
1-Hexadecanol **(13)**	33.78	0.39		$C_{16}H_{34}O$	43 (100) $[C_3H_7]^+$, 41 (92) $[C_3H_5]^+$, 57 (77) $[C_4H_9]^+$, 55 (70) $[C_4H_7]^+$, 82 (60) $[C_6H_{10}]^+$, 69 (41) $[C_5H_9]^+$, 83 (41) $[C_6H_{11}]^+$, 67 (40) $[C_5H_7]^+$, 44 (40) $[C_2H_4O]^+$.
Methyl tetradecanoate **(14)**	33.90	1.03		$C_{15}H_{30}O_2$	242 (3) $[M^+]$, 74 (100) $[C_3H_6O_2]^+$, 87 (63) $[C_4H_7O_2]^+$, 43 (37) $[C_3H_7]^+$, 41 (30) $[C_3H_5]^+$, 55 (27) $[C_4H_7]^+$, 143 (18) $[C_8H_{15}O_2]^+$.
4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol **(15)**	34.39		4.57	$C_{10}H_{12}O_3$	180 (56) $[M^+]$, 137 (100) $[C_8H_9O_2]^+$, 124 (57) $[C_7H_8O_2]^+$, 91 (46) $[C_6H_3O]^+$,77 (29) $[C_6H_5]^+$, 55 (24) $[C_3H_3O]^+$.
4-Acetyl-3-carene **(16)**	34.62		3.55	$C_{12}H_{18}O$	178 (45) $[M^+]$, 43 (100) $[C_2H_3O]^+$, 111 (57) $[C_7H_{11}O]^+$, 41 (54) $[C_3H_5]^+$, 135 (35) $[C_{10}H_{15}]^+$, 67 (33) $[C_5H_7]^+$, 163 (33) $[C_{11}H_{15}O]^+$, 109 (32) $[C_7H_9O]^+$.
Loliolide **(17)**	35.30		15.22	$C_{11}H_{16}O_3$	196 (2) $[M^+]$, 43 (100) $[C_2H_3O]^+$, 111 (76) $[C_7H_{11}O]^+$, 41 (43) $[C_2HO]^+$, 67 (30) $[C_5H_7]^+$, 140 (28) $[C_7H_8O_3]^+$, 107 (27) $[C_6H_3O_2]^+$, 57 (27) $[C_3H_5O]^+$,95 (27) $[C_5H_3O_2]^+$.
4-Hydroxy-3,5,5-trimethyl-4-[3-oxo-1-butenyl]-2-cyclohexen-1-one **(18)**	35.47		4.06	$C_{13}H_{18}O_3$	222 (1) $[M^+]$, 124 (100) $[C_8H_{12}O]^+$, 43 (58) $[C_2H_3O]^+$, 41 (21) $[C_2HO]^+$, 55 (17) $[C_3H_3O]^+$, 69 (13) $[C_4H_5O]^+$, 166 (12) $[C_{10}H_{14}O_2]^+$, 95 (11) $[C_6H_7O]^+$.
Methyl 13-methyltetradecanoate **(19)**	36.07	0.39		$C_{16}H_{32}O_2$	256 (3) $[M^+]$, 74 (100) $[C_3H_6O_2]^+$, 87 (63) $[C_4H_7O_2]^+$, 43 (37) $[C_3H_7]^+$, 41 (33) $[C_3H_5]^+$, 55 (30) $[C_4H_7]^+$.
6,10,14-Trimethyl-2-pentadecanone **(20)**	36.47	0.51		$C_{18}H_{36}O$	268 (1) $[M^+]$, 43 (100) $[C_3H_7]^+$, 58 (63) $[C_3H_6O]^+$, 71 (43) $[C_4H_7O]^+$, 41 (42) $[C_3H_5]^+$, 57 (37) $[C_3H_5O]^+$, 55 (36) $[C_4H_7]^+$, 69 (27) $[C_5H_9]^+$.
3-(4-Hydroxy-3-methoxyphenyl)-2-propenoic acid, methyl ester **(21)**	36.53		1.02	$C_{11}H_{12}O_4$	208 (100) $[M^+]$, 177 (62) $[C_{10}H_9O_3]^+$, 145 (43) $[C_9H_5O_2]^+$, 43 (22) $[C_2H_3O]^+$, 77 (20) $[C_6H_5]^+$, 117 (20) $[C_8H_5O]^+$, 133 (19) $[C_8H_5O_2]^+$, 89 (17) $[C_7H_5]^+$, 105 (17) $[C_7H_5O]^+$.
Neophytadiene **(22)**	36.88	0.26		$C_{20}H_{38}$	278 (5) $[M^+]$, 43 (100) $[C_3H_7]^+$, 82 (91) $[C_6H_{10}]^+$, 81 (86) $[C_6H_9]^+$, 41 (80) $[C_3H_5]^+$, 55 (73) $[C_4H_7]^+$, 57 (70) $[C_4H_9]^+$, 67 (61) $[C_5H_7]^+$, 95 (60) $[C_7H_{11}]^+$, 68 (58) $[C_5H_8]^+$.
1,1-Dimethoxy-dodecane **(23)**	37.07	0.39		$C_{14}H_{30}O_2$	230 (1) $[M^+]$, 75 (100) $[C_3H_7O_2]^+$, 71 (22) $[C_5H_{11}]^+$,41 (17) $[C_3H_5]^+$, 43 (13) $[C_3H_7]^+$, 55 (10) $[C_4H_7]^+$.
Ethyl linoleolate **(24)**	37.63	0.39		$C_{20}H_{36}O_2$	308 (1) $[M^+]$, 79 (100) $[C_6H_7]^+$, 41 (77) $[C_3H_5]^+$, 67 (60) $[C_5H_7]^+$, 55 (50) $[C_4H_7]^+$, 93 (47) $[C_7H_9]^+$, 95 (47) $[C_7H_{11}]^+$, 91 (33) $[C_7H_7]^+$.
Methyl Z-9-hexadecenoate **(25)**	37.74	0.64		$C_{17}H_{32}O_2$	268 (2) $[M^+]$, 55 (100) $[C_4H_7]^+$, 41 (82) $[C_3H_5]^+$, 69 (66) $[C_5H_9]^+$, 43 (60) $[C_3H_7]^+$, 74 (53) $[C_3H_6O_2]^+$, 67 (40) $[C_5H_7]^+$, 83 (39) $[C_6H_{11}]^+$.
Methyl palmitate **(26)**	38.16	20.08	0.51	$C_{17}H_{34}O_2$	270 (3) $[M^+]$, 74 (100) $[C_3H_6O_2]^+$, 87 (72) $[C_4H_7O_2]^+$, 43 (39) $[C_3H_7]^+$, 41 (31) $[C_3H_5]^+$, 55 (30) $[C_4H_7]^+$.
trans-Sinapyl alcohol **(27)**	39.54		2.03	$C_{11}H_{14}O_4$	210 (100) $[M^+]$, 55 (99) $[C_3H_3O]^+$, 41 (95) $[C_2HO]$, 167 (94) $[C_9H_{11}O_3]^+$, 43

					(94) $[C_2H_3O]^+$, 69 (59) $[C_4H_5O]^+$, 77 (53) $[C_6H_5]^+$, 57 (53) $[C_3H_5O]^+$, 91 (43) $[C_6H_3O]^+$, 97 (43) $[C_5H_5O_2]^+$.
Methyl 15-methylhexadecanoate **(28)**	40.11	0.51		$C_{18}H_{36}O_2$	284 (3) $[M^+]$, 74 (100) $[C_3H_6O_2]^+$, 87 (70) $[C_4H_7O_2]^+$, 43 (47) $[C_3H_7]^+$, 41 (38) $[C_3H_5]^+$, 55 (34) $[C_4H_7]^+$.
Methyl sinapate **(29)**	41.34		2.54	$C_{12}H_{14}O_5$	238 (100) $[M^+]$, 207 (33) $[C_{11}H_{11}O_4]^+$,163 (23) $[C_9H_7O_3]^+$, 43 (17) $[C_2H_3O]^+$, 175 (15) $[C_{10}H_7O_3]^+$, 135 (15) $[C_8H_7O_2]^+$, 91 (13) $[C_6H_3O]^+$.
Methyl linoleate **(30)**	41.45	13.90		$C_{19}H_{34}O_2$	294 (1) $[M^+]$, 67 (100) $[C_5H_7]^+$, 81 (80) $[C_6H_9]^+$, 55 (67) $[C_4H_7]^+$, 41 (60) $[C_3H_5]^+$, 95 (53) $[C_7H_{11}]^+$, 79 (45) $[C_6H_7]^+$.
4-Hexadecen-6-yne **(31)**	41.55		1.01	$C_{16}H_{28}$	220 (1) $[M^+]$, 79 (100) $[C_6H_7]^+$, 41 (97) $[C_3H_5]^+$, 55 (90) $[C_4H_7]^+$, 67 (87) $[C_5H_7]^+$, 43 (79) $[C_3H_7]^+$, 91 (66) $[C_7H_7]^+$, 93 (63) $[C_7H_9]^+$, 81 (57) $[C_6H_9]^+$.
Methyl linolenate **(32)**	41.58	27.02		$C_{19}H_{32}O_2$	292 (1) $[M^+]$, 79 (100) $[C_6H_7]^+$, 41 (87) $[C_3H_5]^+$, 67 (84) $[C_5H_7]^+$, 55 (71) $[C_4H_7]^+$, 43 (57) $[C_2H_3O]^+$, 95 (48) $[C_7H_{11}]^+$, 93 (47) $[C_7H_9]^+$, 81 (47) $[C_6H_9]^+$.
Phytol **(33)**	41.76	5.28		$C_{20}H_{40}O$	296 (1) $[M^+]$, 71 (100) $[C_4H_7O]^+$, 43 (48) $[C_3H_7]^+$, 41 (37) $[C_3H_5]^+$, 57 (36) $[C_4H_9]^+$, 55 (35) $[C_4H_7]^+$, 69 (29) $[C_5H_9]^+$, 81 (27) $[C_6H_9]^+$.
Methyl stearate **(34)**	41.99	4.63		$C_{19}H_{38}O_2$	298 ($[M^+]$, 74 (100) $[C_3H_6O_2]^+$, 87 (71) $[C_4H_7O_2]^+$, 43 (43) $[C_3H_7]^+$, 55 (30) $[C_4H_7]^+$, 41 (29) $[C_3H_5]^+$, 57 (20) $[C_4H_9]^+$.
Methyl eicosanoate **(35)**	45.52	1.67		$C_{21}H_{42}O_2$	326 (12) $[M^+]$, 74 (100) $[C_3H_6O_2]^+$, 87 (73) $[C_4H_7O_2]^+$, 43 (57) $[C_3H_7]^+$, 55 (48) $[C_4H_7]^+$, 41 (45) $[C_3H_5]^+$, 57 (30) $[C_4H_9]^+$.
14-Methylpregn-4-ene-3,20-dione **(36)**	52.50	6.05		$C_{22}H_{32}O_2$	328 (24) $[M^+]$, 43 (100) $[C_2H_3O]^+$, 124 (38) $[C_8H_{12}O]^+$, 123 (38) $[C_8H_{11}O]^+$, 91 (36) $[C_7H_7]^+$, 79 (28) $[C_5H_3O]^+$.

Repeated chromatographic separations of the methylene chloride fraction have resulted in isolation and purification of compound **(38)** which was elucidated by means of complete ^1H and ^{13}C NMR spectral analyses. The ^1H NMR spectrum of **(38)** showed two methyl singlets, at δ 0.92 (3H, s), 1.22 (3H, s) and one acetyl singlet at δ 2.38 (3H, s), which suggested the presence of a pregnane steroidal skeleton.

Two olefinic protons are clearly parts of *α, β*-unsaturated carbonyl systems were characterized, an α proton to the carbonyl groups resonated at δ 5.75 (1H, s) while the other *β* proton system appeared at 6.97 (1H, d, J 3.1 Hz), in addition to a carbinylic proton at δ 3.68 (1H, dd, J 10.6, 5.5 Hz), indicating the presence of *β*-hydroxyl group. The ^{13}C NMR spectrum of **(38)** showed the presence of signals for three tertiary methyl groups (δ_C 11.7, 16.9 and 32.7), two carbonyl carbons at (δ_C 199.0 and 199.1), four olefinic carbons (δ_C 124.6, 170.2, 149.7 and 155.2) and one oxy carbon at δ_C 73.3 ppm.

All the above detailed observations revealed the structure of **(38)** to be 12*β*-hydroxy-4,16-pregnadien-3,20-dione which was earlier reported from *Thevetia neriifolia* [20] and reported from this species for the first time.



38

Experimental

General

NMR spectra were recorded on 500 MHz (JEOL). Chemical shifts are given in *δ* (ppm) relative to TMS as internal standard material at Faculty of Science, Mansoura University. GC/MS analysis of the volatile fractions were performed on a Varian GC interfaced to Finnegan SSQ 7000 Mass selective Detector (SMD) with ICIS V2.0 data system for MS identification of the GC components. The column used was DB-5 (J&W Scientific, Folosm, CA) cross-linked fused silica capillary column (30 m. long, 0.25 mm. internal diameter). The oven temperature was programmed from 50°C for 3 min. Injector temperature was 200°C and the volume injected was 0.5 µl. Ionization energy was set at 70 eV. (Agriculture Research Center (NRC), Dokki, Cairo).

Chemicals

Thin layer chromatography and preparative (TLC) were performed on silica gel (Kieselgel 60, GF 254) of 0.25 thickness. Petroleum ether (60-80), methylene chloride, methanol and anhydrous sodium sulphate were purchased from Adwic Company.

Plant material

Thevetia peruviana leaves was collected from Mit Ghamr, Dakahlia Governorate, Egypt on May, 2016. The authenticity of the plant was confirmed kindly by the staff of department of Horticulture, Faculty of Agriculture, Mansoura University.

Extraction process

The air-dried leaves of *T. peruviana* (1kg) were soaked in methanol (4 x 3 L) at room temp. The methanolic extract was filtered, concentrated and exhaustively partitioned successively with pet. ether and methylene chloride to furnish pet. ether (8 g) and methylene chloride (0.8 g) fractions. The pet. ether fraction was defatted using cold methanol, then a portion of the resulting both fractions were subjected to GC/MS analysis.

The resulting methylene chloride fraction (0.8 g) was purified by repetitive PTLC using (silica gel, pet. ether/ethyl acetate 9:1) to obtain12*β*-hydroxy-4,16-pregnadien-3,20-dione **(38)** (16 mg, R*f* 0.36)

12*β*-Hydroxy-4,16-pregnadien-3,20-dione: White crystals, ^{1}H NMR ($CDCl_3$, 500 MHz): δ_H 2.34 (2H, m, H-2), 5.75 (1H, s, H-4), 3.68 (1H, dd, J 10.6, 5.5 Hz, H-12), 2.25 (1H, ddd, J 17, 12, 2 Hz, H-15_a), 2.42 (1H, m, H-15_b), 6.97 (1H, dd, J 3.1, 2 Hz, H-16), 0.92 (3H, s, H-18), 1.22 (3H, s, H-19), 2.38 (3H, s, H-21), 5.84 (1H, s, -OH). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 32.6 (C-1), 34.0 (C-2), 199.1(C-3), 124.6 (C-4), 170.2 (C-5), 31.5 (C-6), 29.8 (C-7), 27.1 (C-8), 52.7 (C-9), 38.9 (C-10), 35.6 (C-11), 73.3 (C-12), 52.0 (C-13), 53.4 (C-14), 29.2 (C-15), 149.7 (C-16), 155.2 (C-17), 11.7 (C-18), 16.9 (C-19), 199.0 (C-20), 32.7 (C-21).

4. References

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