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Phytochemical Investigation Of Aerial Parts Of Canna indica Collected From Uttarakhand India

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Abstract: *Canna indica* is an erect perennial herb widely cultivated throughout India for its beautiful foliage and flowers. The roots are used as diuretic, demulcent, diaphoretic and also in dropsy and fever. The infusion of the leaves is reported to possess diuretic property and is used in fever .The plant is reported to possess molluscidal and anti-fungal activities.Chemical analysis of aerial parts of *Canna indica* afforded Betulinic acid, Oleonolic acid and Traraxer-14-en-3-one.The structures of these compounds were established by extensive use of IR, UV, NMR spectroscopy and chemical method.
Key words: *Canna indica,* IR, UV, NMR.

Introduction

Canna Linn. (Cannaceae) is a genus of herbs with rhizomatous root stocks, distributed in the tropics and sub-tropics particularly of the western hemisphere. About 15 species are found in India. Several hybrids of canna species are grown in garden mainly for their decorative foliage and showy flowers.*Canna indica* L. is commonly known as Indian shot or Canna lily. Several varieties are common all over India and are grown in gardens. It is extensively used in constructed wetland for removal of organic pollutants, nitrogen, phosphorous and heavy metals[1-2]. It is an upright perennial rhizomatous herb up to 5 feet high, whose leaves are fleshy with thin margins, usually not more than 1 foot long and half as broad, lanceolate to sub-orbicular. The flowers are red or yellow and showy. It encloses a variable number of round, shiny black seeds. In folkloric medicine, root decoction is used for the treatment of fever, dropsy, and dyspepsia. Seed juice is used to relieve earaches. The flowers are said to cure eye diseases[3-4]. The leaves of *C. indica* showed antimicrobial activity[5], analgesic activity, and the rhizomes showed a good anthelmintic activity against *Pheritima posthuma*[6]. The flower are said to cure eye diseases[7] and shows antibacterial activity[8] Flowers contain lutein, ß – carotene, violxanthin. Its leaves haves chemical constituents like lignin, furfural,hemicelluloses.While rhizomes has 5,8-Henicosdine,Tetracosane,Tricosane[9-10]. The water extract of rhizomes of *C. indica* has been reported to have HIV-1reverse transcriptase inhibitory activity[11] while its essential oil shows antibacterial activity[12]. Methanolic extract of Aerial Parts of *Canna indica* shows Antioxidant Activity[13]. Anthocyanins and methylated anthocyanidin glycosides were also isolated from *canna indica* flowers[14-15].

Material And Method

General

CC was carried out over silica gel (60-120 mesh BDH) and Sephadex LH-20 (25-100μ, Pharmacia) using gradient elution method with different solvent systems in order of increasing polarity. TLC was conducted on SI-gel (E-Merck and BDH) coated on a thin glass plate (0.25 mm thickness containing 13% $CaSO_4$ as binder). Spots on TLC were detected by spraying with 5% H_2SO_4 followed by heating at 100^0C, 5% methanolic KOH, Benedict's reagent, iodine vapours, UV and alcoholic $FeCl_3$ solution. PC was carried out on Whatman filter paper No. 1 using descending with n-butanol-pyridine-water (6:4:3) as solvent system and spots were detected by spraying with aniline hydrogen phthalate (AHP) followed by heating. MPs were recorded in BOETIUS microscopic M P apparatus. Optical rotations were recorded in methanol on JASCO DIP-140 digital and Autopol II polarimeter. UV-spectra (λ_{max}, nm) were recorded in MeOH on a BECKMAN DU-64 spectrophotometer. IR-spectra (ν_{max}, cm^{-1}) were carried out on SP-3-200 PYE UNICAM, and FT-IR-8100 Shimadzu spectrophotometer as KBr palettes.

Plant Material

The whole plants of *Canna indica* were collected from Forest Research Institute Campus, New Forest, Dehradun, Uttarakhand India.The plant species were identified by Dr. Sumer Chand, Scientist, Systematic Botany Division, Forest Research Institute, Dehradun, India

Extraction and Isolation

The air-dried and powdered whole plant (1.5 kg) was exhaustively defatted with light petroleum ether ($60-80^0$). The petroleum free mass was then extracted with 90% aqueous ethanol. The ethanol extract (15 g) was concentrated under reduced pressure in vacuum and dried. A suspension of the residue was made with water, which was washed with diethyl ether for several times and then partitioned with $CHCl_3$:H_2O:MeOH (6:4:4) in a separatory funnel. The chloroform layer was separated out and concentrated under reduced pressure to give $CHCl_3$ extract. The aqueous layer was successively extracted with EtOAc and BuOH (saturated with water). The EtOAc and BuOH layers were concentrated under reduced pressure to give EtOAc and BuOH extracts respectively. The BuOH extract was further digested with methanol:water (8:2) and filtered. The filtrate was evaporated to dry under reduced pressure to give methanol extract.

The chloroform extract, ethyl acetate extract and MeOH extract were subjected to column chromatography over various adsorbents with various solvents in order to their increasing polarity

Ethyl Acetate Extract

The ethyl acetate extract (8.0 g) was subjected to CC over Si-gel using gradient elution with $CHCl_3$:MeOH (10:0 → 9:1) to get various fractions. The $CHCl_3$:MeOH (95:5) fraction (4.0 g) was subjected to CC over Si-gel using gradient elution with $CHCl_3$:MeOH (98:2→90:10) afforded compound **1** (150 mg) and various other fractions. The fractions obtained $CHCl_3$:MeOH (95:5) were mixed and evaporated to dryness. The extract obtained was further subjected to CC over Si-gel eluted with $CHCl_3$:MeOH (95:5) afforded compound 2 (200 mg) and compound **3**(83 mg).

Result and Discussion

COMPOUND: 1

It was crystallized from Chloroform as yellow powder M.P. 295-297°C.

Elemental Analysis: Found values C=78.41%, H=10.15%; required values for $C_{30}H_{48}O_3$; C=78.95%, H=10.53%; Molecular weight 456.

FAB^+-MS: m/z 457 $[M]^+$, 439, 437, 411, 391, 379, 307, 289, 242, 220

IR (V_{max}^{KBr}): cm^{-1} 3400-3280, 2900, 1715, 1635, 1450, 1375, 1360, 1045, 1010 etc.

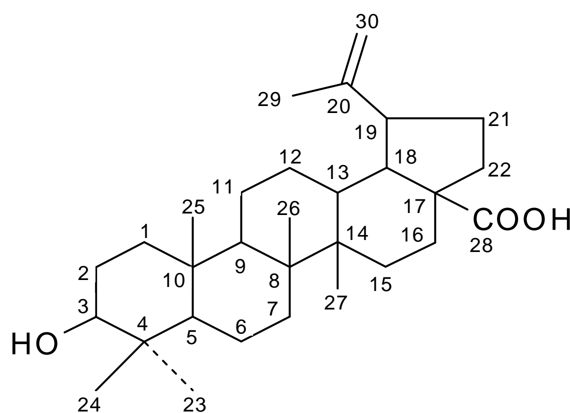
^{1}H-NMR: (400 MHz, DMSO): 0.65, (3H, *s*), 0.77 (3H, *s*), 0.87 (6H, *s*), 0.93 (3H, *s*), 1.65 (3H, *s*), 0.98-1.60 (*m*), 4.69 (1H, *brs*, H-30a) 4.57 (1H, *brs*, H-30b) 3.31 (1H, *d*, *J* = 7.4, H-3) 2.96 (1H, *d*, *J* = 10.0 Hz, H-16a) 2.22 (1H, *t*), 2.12 (1H, *d*) 1.80 (1H, *d*, *J* = 10.0 Hz, H-16a)

^{13}C-NMR (100 MHz, DMSO): 38.3 (t, C-1), 25.0 (t, C-2), 76.8 (d, C-3), 37.6 (s, C-4), 54.9 (d, C-5), 17.9 (t, C-6), 33.9 (t, C-7), 40.2 (s, C-8), 49.9 (d, C-9), 36.3 (s, C-10), 29.2 (t, C-11), 27.1 (d, C-12), 38.5 (s, C-13), 41.9 (s, C-14), 20.4 (t, C-15), 31.7 (t, C-16), 55.4 (s, C-17), 48.5 (d, C-18), 46.6 (d, C-19), 150.3 (s, C-20), 30.1 (t, C-21), 36.7 (t, C-22), 28.1 (q, C-23), 15.8 (q, C-24), 15.9 (q, C-25), 15.8 (q, C-26) 14.4 (q, C-27), 177.2 (s, C-28), 18.9 (q, C-29), 109.6 (t, C-30) Multiplicitiy of the signals was given by DEPT.

Elemental analysis of compound **1** corresponded to molecular formula $C_{30}H_{48}O_3$ which was confirmed by the presence of molecular ion peak $[M]^+$ at m/z 457 in FAB-positive mass spectrum. It gave deep red colour with Liebermann-Burchard reagent the characteristics of sterols. It gave yellow colour with TNM reagent indicating unsaturation in the molecule. It did not respond to Molisch's reagent indicating non-glycosidic nature of the molecule. The IR spectrum showed the presence of hydroxyl group (3400), carboxylic carbonyl group (1715), unsaturation (1635), and gem-dimethyl (1375, and 1360 cm^{-1}), which led to the conclusion that the molecule was triterpenoid containing a carboxylic functional group.

The ^{1}H-NMR spectrum of compound **1** displayed presence of six methyl groups at δ 0.65, (3H, *s*, Me), 0.77 (3H, *s*, Me), 0.87 (6H, *s*, 2-Me), 0.93 (3H, *s*, Me), and 1.65 (3H, *s*, Me). Presence of a terminal methylene group was determined by the two broad singlets each for one proton at δ 4.69 and 4.57 which was confirmed by ^{13}C-chemical shifts of double bonded carbon atoms at δ 109.6 (C-30) and 150.3 (C-20) in ^{13}C-NMR spectrum (16). The ^{13}C-NMR spectrum of compound **1** displayed presence 30 carbon atoms in the molecule. The DEPT spectrum showed presence of six methyl, eleven methylene, six methine and seven quaternary carbon atoms in the compound. The ^{13}C-NMR spectrum also displayed presence of a carboxyl carbon atom at δ 177.2, an oxygenated carbon atom at δ 76.8 and six methyl carbon at δ 28.1 (C-23), 15.8 (C-24), 15.9 (C-25), 15.8 (C-26) 14.4 (C-27), and 18.9 (C-29). It gave an acetyl derivative with pyridine-acetic anhydride, m.p. 282-284^{0}C, indicating the presence of an OH group in molecule.

On the basis of above discussed spectral and chemical evidences compound **1** was identified as betulinic acid. The identity of the compound was finally confirmed by MMP, co-TLC and co-IR with an authentic sample and by comparing the ^{13}C-NMR data with literature[16].



COMPOUND: 2

It was crystallized from MeOH as white needles, M.P. 305-306°C.

Elemental Analysis: Found values C=78.46%, H=10.58%; required values for $C_{30}H_{48}O_3$; C=78.94%, H=10.52%; Molecular weight 456.

EI$^+$-MS: m/z 456 $[M]^+$, 441, 430, 411, 400, 391, 329, 325, 307, 289, 271, 248, 207, 203, 189, 133, 107

IR (V_{max}^{KBr}): cm^{-1} 3400, 3120, 2920, 2880, 1690, 1450, 1375, 1360, 1045, 1010 etc.

^{1}H-NMR: (400 MHz, DMSO): δ, (ppm) 5.27 (1H, t, J = 4.0 Hz, H-12), 4.50 (1H, t, J = 5.2 Hz, H-3), 0.83, 0.85, 0.88, 0.91, 0.93, 0.95, (each 3H s), 1.21 (3H, s), 2.38 (2H, dd, J = 4.0 and 8.0 Hz), 2.75 (2H, dd, J = 2.0 and 11.6 Hz), 1.90 (2H, m), 0.95-1.78 (m)

^{13}C-NMR: (100 MHz, DMSO): δ, (ppm) 38.27 (t, C-1), 23.05 (t, C-2), 80.6 (d, C-3), 37.59 (s, C-4), 55.4 (d, C-5), 18.14 (t, C-6), 31.72 (t, C-7), 40.04 (s, C-8), 47.6 (d, C-9), 36.70 (s, C-10), 22.02 (t, C-11), 122.56 (d, C-12), 143.78 (s, C-13), 41.99 (s¸C-14), 27.70 (t, C-15), 23.45 (t, C-16), 46.6 (s, C-17), 40.26 (d, C-18), 45.54 (t, C-19), 30.12 (s, C-20), 33.90 (t, C-21), 32.52 (t, C-22), 28.08 (q, C-23), 16.45 (q, C-24), 15.36 (q, C-25), 17.16 (q, C-26), 25.84 (q, C-27), 184.79 (s, C-28), 33.02 (q, C-29), 24.04 (q, C-30) . Multiplicity of carbon signals was given by DEPT

The molecular formula of compound **2** was determined to be $C_{30}H_{48}O_{3,}$ by elemental analysis, which corresponded to the molecular weight 456. The molecular weight determined by elemental analysis was substantiated by EI-mass spectrum which displayed molecular ion peak $[M]^+$ at m/z 456. It gave positive LB and Noller test and developed yellow colour with TNM indicating triterpenoid nature of the molecule. It did not respond Molisch's test showing non-glycosidic nature of the molecule.

The IR spectrum of compound **2** exhibit characteristic absorption band for -OH function at 3400 cm^{-1}, for OH of carboxylic function at 3120 cm^{-1}, C-H stretching at 2920, and 2880 cm^{-1}, for carboxylic function at 1690 cm^{-1}, and presence of double bond at 1450 cm^{-1}.

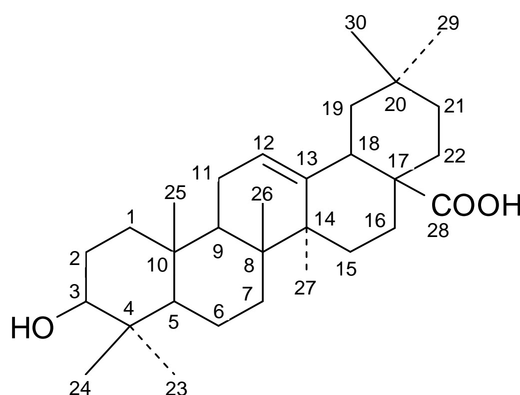
The ^{1}H-NMR spectrum of compound **2** exhibit presence of seven methyl groups at δ 0.83, 0.85, 0.88, 0.91, 0.93, 0.95, and 1.21 and a characteristic olefinic proton of C_{12}-C_{13} double bonded pentacyclic-triterpenoid at δ 5.27 (1H, *t*, *J* = 4.0 Hz, H-12)**[17]**. The location of the double bond at C_{12}-C_{13}-position was supported by the diagnostic mass spectral fragmentation pattern**[18]** exhibited by compound **2** forming ion peak at m/z 289. The fragment ion peaks at m/z 248 $[C_{16}H_{24}O_2]^+$, 203 $[C_{15}H_{23}]^+$. 189 $[C_{14}H_{21}]^+$ and 133 $[C_{10}H_{13}]^+$ derived from D, E rings via retro-Diels-Alder fission indicating that compound **2** possesses one hydroxyl group in A, B-ring and a carboxyl group in the D, E-ring on the olean-12-ene skeleton**[19]**.

The ^{1}H-NMR spectrum also showed a downfield signal for oxygenated methine proton at δ 4.50 (1H, *t*, *J* = 5.2 Hz,), which was assigned for H-3 proton. The ^{13}C-NMR spectrum of compound **2** revealed presence of signals due to a oxygenated carbon signal at δ 80.6 (C-3), one tri-substituted double bond at δ 122.56 (C-12) and 143.78 (C-13) and one carboxyl group at 184.79 (C-28). Moreover ^{13}C-NMR signals due to C_{-18}-C_{-22} [40.26 (C-18), 45.54 (C-19), 30.12 (C-20), 33.90 (C-21), and 32.52 (C-22)] suggested that compound 2 was an olean-12-en derivative**[20]**. It forms monoacetate with acetic anhydride-pyridine suggesting the presence of one hydroxyl group in the molecule.

The DEPT spectrum of compound **2** displayed presence of seven methyl, ten methylene, five methine and eight quaternary carbon atoms in molecule.

The IR spectral data coupled with mass and ^{13}C-spectral data collectively confirmed the presence of one hydroxyl group, one carboxylic group and seven-methyl groups [28.08 (C-23), 16.45 (C-24), 15.36 (C-25), 17.16 (C-26), 25.84 (C-27), 33.02 (C-29), and 24.04 (C-30)] in an olean-12-en skeleton.

On the basis of above spectral and chemical evidences compound **2** was identified as oleonolic acid. The identity of the compound was finally determined by co-TLC, and MMP with an authentic sample and by comparison of ^{13}C-chemical shifts with the reported data**[21]**.



COMPOUND: 3

It was crystallized from MeOH as white solid, M.P. 150-151^{0}C, $[\alpha]_D$-34^0 (c, 0.1 in $CHCl_3$).

Elemental Analysis: Found values, C=84.96%, H=11.42%, required values for $C_{30}H_{48}O$; C=84.91%, H=11.32%; Molecular weight 424.

MS-EI$^+$: m/z 424 $[M]^+$, 409, 396, 204, 147, 135, 91

IR (ν_{max}^{KBr}): cm^{-1} 3000, 2949, 1715, 1640, 1390, 1380, 1260, 1210, 1155,1010, 990 etc.

^{1}H-NMR (400 MHz, C_5D_5N):

^{13}C-NMR (100 MHz, C_5D_5N):

Compound **3** was crystallized as white solid from MeOH, m.p. 150-151^{0}C. It gave red colour with Liebermann-Burchard reagent suggesting that the compound is a triterpenoid[22-23]. The molecular formula of compound **3** was determined to be $C_{30}H_{48}O$, by elemental analysis, which corresponded to the molecular weight 424. The molecular weight determined by elemental analysis was substantiated by EI-mass spectrum which displayed molecular ion peak $[M]^+$ at m/z 424. It did not respond to Molisch's test showing non-glycosidic nature of the molecule.

The IR spectrum of compound **3** exhibit characteristic absorption band for carbonyl function at 1715 cm^{-1}, C-H stretching at 3000, and 2949 cm^{-1}, gem di-methyl group at 1390 and 1380 cm^{-1}, and presence of double bond at 1640 cm^{-1}.

The ^{1}H-NMR spectrum of compound **3** exhibit presence of eight methyl groups at δ 0.91, 0.98, 0.99, 1.00, 1.02, 1.06, 1.12 and 1.17 and a characteristic olefinic proton of C_{14}-C_{15} double bonded pentacyclic-triterpenoid at δ 5.61 (1H, *dd*, *J* = 3.2 and 8.0 Hz, H-12) (24 – 25) The ^{13}C-NMR spectrum of **6** revealed presence of signals due to a carbonyl carbon at δ 215.9 (C-3), and one tri-substituted double bond at δ 157.9 (C-14) and 117.0 (C-15). Moreover ^{13}C-NMR signals due to C-18-C-22 [49.1 (C-18), 36.9 (C-19), 29.0 (C-20), 37.6 (C-21), and 35.3 (C-22)] suggested that **3** was an olean-14-en derivative[23-25]. It did not forms monoacetate with acetic anhydride-pyridine suggested the absence of hydroxyl groups in the molecule.

The DEPT spectrum of compound **3** displayed presence of eight methyl, ten methylene, three methine and nine quaternary carbon atoms in molecule. The assignment of proton and carbon signals was made by 2D-NMR experiments, ^{1}H-^{1}H-COSY and ^{1}H-^{13}C-COSY.

The absolute structure of the molecule was determined by ^{1}H-^{13}C heteronuclear multiple bond correlation (HMBC) experiment. The methyl group at δ 1.06 (H-23) showed $^2J_{CH}$ correlation with C-4, and $^3J_{CH}$ correlation with C-24, C-3 (carbonyl carbon), and C-5 while the methyl singlet at δ 1.17 (H-24) showed $^2J_{CH}$ correlation with C-4, and $^3J_{CH}$ correlation with C-23, and C-3. These HMBC correlations coupled with the ^{1}H- and ^{13}C-chemical shifts of proton and carbon assigned for position 4, 23 and 24 confirmed that gem-methyl group was attached at C-4 carbon atom and the carbonyl function at C-3.

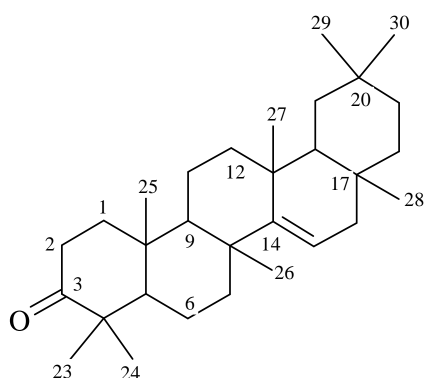
Table.1: 500 MHz ^{1}H-NMR and 125 MHz ^{13}C-NMR data (in ppm relative to TMS) of compound **3** in C_5D_5N.

C/H	^{u}C	DEPT	^{u}H (*J* in Hz)	HMBC Correlation (H→→C)
1	38.4	CH_2	1.22 (1H, *m*); 1.67 (1H, *m*)	C-2, C-3
2	33.8	CH_2	1.52 (*m*), 1.64 (*m*)	C-3
3	215.9	C	--	--
4	47.5	C	--	--
5	55.7	CH	1.27 (1H, *m*)	C-6, C-4
6	17.7	CH_2	1.47 (2H, *m*)	C-5, C-7
7	40.8	CH_2	1.33 (1H, *m*); 2.02 (1H, *m*)	C-6, C-8
8	39.1	C	--	--
9	48.8	CH	1.47 (1H, *m*)	C-10, C-8, C-11
10	37.8	C	--	--
11	20.1	CH_2	1.46 (1H, *m*); 2.60 (1H, *m*)	C-12, C-9

12	34.3	CH_2	2.37 (1H, *ddd,* 7.0, 11.0, 15.8)	C-13, C-9
			2.60 (1H, *ddd,* 7.0, 11.0, 15.8)	C-9, C-14
13	37.7	C	--	--
14	157.9	C		--
15	117.0	CH	5.61 (1H, *dd*, 3.2, 7.9)	C-8, C-9, C-17,
16	37.9	CH_2	1.69 (1H, *m*); 2.01 (1H, *m*)	C-15, C-16, C-28
17	36.0	C	--	--
18	49.1	C	1.30 (1H, m)	C-17
19	36.9	CH_2	1.03 (1H, *m*); 1.38 (1H, *m*)	C-20
20	29.0	C	--	--
21	37.6	CH_2	1.04 (H, m)	C-20
22	35.3	CH_2	1.05 (1H, *m*); 1.42 (1H, *m*)	C-17
23	21.6	CH_3	1.06 (3H, *s*)	C-3, C-4, C-5, C-24
24	26.3	CH_3	1.17 (3H, *s*)	C-3, C-4, C-5, C-23
25	14.8	CH_3	1.00 (3H, *s*)	C-1, C-9, C-10
26	25.8	CH_3	1.12 (3H, *s*)	C-5, C-7, C-8, C-14
27	21.5	CH_3	0.98 (3H, *s*)	C-10, C-12, C-14, C-18
28	30.2	CH_3	0.91 (3H, *s*)	C-16, C-17, C-18, C-22
29	33.4	CH_3	1.02 (3H, *s*)	C-19, C-20, C-21, C-30
30	30.0	CH_3	0.99 (3H, *s*)	C-19, C-20

The position of carbonyl function at C-3 was further confirmed by $^3J_{CH}$ correlation of protons resonating at δ 1.22 & 1.67 (H-1) and $^2J_{CH}$ correlation of protons resonating at δ 1.52 and 1.64 (H-2) protons with carbonyl carbon (δ 215.9, C-3). Similarly, the methyl group at δ 1.02 (H-29) showed $^2J_{CH}$ correlation with C-20, and $^3J_{CH}$ correlation with C-19, C-21 and C-30, while the methyl singlet at δ 0.99 showed $^2J_{CH}$ correlation with C-20 and $^3J_{CH}$ correlation with C-19, which indicate that another gem-methyl group was attached at C-20 carbon atom. The $^2J_{CH}$ correlations of methyl proton (above table) resonating at δ 1.00 (H-25), 1.12 (H-26), 0.98 (H-27), and 0.91 (H-28) with C-10, C-8, C-13, and C-17, respectively, confirmed that the methyl groups were attached with these carbon atoms. The HMBC correlations of olifinic proton (δ5.61, H-15) with C-8, C-13 and C-17, confirmed the position of double bond at C-15. The other long-range correlations identified by HMBC are given in table No.1.

On the basis of above discussed spectral data and chemical evidences compound 3 was identified as traraxer-14-en-3-one.



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