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Constituents of *Nepeta crassifolia* (Lamiaceae)

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A long chain ketone (crassifone), a pentacyclic triterpenoid coupled with fatty acid moiety (crassifoate), and an acyclic diterpenoid (crassifol) have been isolated from the ethanol soluble part of *Nepeta crassifolia* (Lamiaceae) collected from Kangavar, Iran. Structures of all the metabolites were elucidated with the aid of spectroscopic techniques, including 2D NMR experiments.

Key Words: *Nepeta crassifolia*, Lamiaceae, spectroscopic characterization, crassifone, crassifoate, crassifol.

Introduction

Nepeta is a multiregional genus of the family Lamiaceae (Labiatae), comprising about 250 species distributed mainly in southwest and central Asia, Europe, North Africa, and North America.[1,2] Several species of the genus *Nepeta* are rich in interesting biological activities and, for this reason only, many members of *Nepeta* have been investigated for bioactive constituents.[3] Among the various medicinal properties, *Nepeta* species are famous for treating cardiovascular complaints, such as angina pectoris, cardiac thrombosis, tachycardia, and weakness of the heart.[4–6] Several Iranian *Nepeta* species have been of great interest for use in Iranian folk and traditional medicines, and are used in the treatment of various diseases,[7] including *N. hindostana* for sore throat[8] and its decoction for fever and pain, including ear and tooth aches.[9] *N. glomerulosa* is used to treat digestive troubles, pneumonia, and itching.[10] Most *Nepeta* plants are rich in essential oils and, among their constituents, triterpenes are the most common.[11–14]

The present work describes the isolation and characterization of 3 new constituents: a long chain ketone (**1**, crassifone), a fatty acid coupled pentacyclic triterpene (**2**, crassifoate), and an acyclic diterpene (**3**, crassifol). The structures of **1-3** were elucidated by spectroscopic techniques, including modern NMR experiments.

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Experimental

General techniques

Melting points were determined on a Gallenkamp apparatus and are uncorrected. Optical rotations were measured on a JASCO DIP-360 digital polarimeter in $CHCl_3$ using a 10-cm cell tube. IR spectra were recorded on a Jasco-320-A spectrometer. Mass spectra (EI, FD, and HR-EI) were measured in an electron impact mode on a Finnigan MAT 12 or MAT 312 spectrometer, and ions are given in m/z (%). ^{1}H- and ^{13}C-NMR spectra were recorded in $CDCl_3$ with a Bruker AM-400 spectrometer at 400 MHz and at 100 MHz. Chemical shifts are given relative to TMS as an internal standard. TLC was performed with precoated silica gel G-25-UV_{254} plates and detection was made by spraying with ceric sulphate in 10% H_2SO_4. Silica gel (E. Merck, 230-400 mesh) was used for column chromatography.

Plant material

Nepeta crassifolia (all parts) was collected from Kangavar, Iran, in August 2003 and identified by Prof. Sanei Chariat Pannahi at Karaj Agriculture College, University of Tehran, where voucher specimens of the collected plant material are deposited in the herbarium.

Extraction and isolation

Shade-dried *Nepeta crassifolia* (2 kg, whole plant) was soaked in ethanol (5 L) for 10 days. The ethanol was evaporated under reduced pressure to avoid thermal decomposition. The crude ethanol soluble part thus obtained (71 g) was subjected to vacuum-liquid chromatography (VLC) using flesh silica (Merck, mesh size: 230-400 μm). Elution was carried out using 20%, 40%, 60%, and 80% ethyl acetate in hexane, and finally pure ethyl-acetate as the mobile phase. The fraction obtained with 20% ethyl acetate in hexane was re-chromatographed on a silica gel column using hexane, hexane-chloroform, and finally pure chloroform as the mobile phase, yielding compounds **1**- **3**.

Fraction eluted with 20% chloroform in hexane yielded **1** as a white solid (15 mg).

Crassifone (1)

mp: 164 °C; IR ($CHCl_3$): 1725 (ketone C=O) cm^{-1}; EIMS: m/z 492 $[M]^+$, 477 $[M\text{-}CH_3]^+$, 449 $[M\text{- }COCH_3]^+$; HR-EIMS: m/z 492.5273 ($C_{34}H_{68}O$ requires m/z 492.5269), 477. 5039 ($C_{33}H_{65}O$ requires m/z 477.5035), 449.5090 ($C_{32}H_{65}$ requires m/z 449.5086); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 2.39 (2H, t, J = 7.4 Hz, H-3), 2.11 (3H, s, H-1), 1.54 (2H, m, H-4), 1.24 (br. s, chain), and 0.86 (3H, t, J = 6.9 Hz, H-34); ^{13}C-NMR: ($CDCl_3$, 100 MHz): δ 29.8 (C-1), 209.5 (C-2), 43.8 (C-3), 23.9 (C-4), 29.2 (C-31), 31.9 (C-32), 22.7 (C-33), 14.1 (C-34), and 29.3-29.7(chain); HMBC: See Figure 1.

Fraction eluted with 25% chloroform in hexane yielded **2** as a white solid (10 mg).

Crassifoate (2)

mp: 112 °C; $[\alpha]_D^{28}$ = –76° (chloroform, c 0.665); IR ($CHCl_3$): 1735 (ester C=O), 1640 (C=C), 1190 (C–O) cm^{-1}; EIMS: m/z 706 $[M]^+$, 423 $[M\text{-stearyl}]^+$, 405 $[M\text{-stearyl} + H_2O]^+$, 299 [rDA], and 283 $[stearyl]^+$; HR-EIMS: m/z 706.6267 ($C_{48}H_{82}O_3$ requires m/z 706.6263), 423.3631 ($C_{30}H_{47}O$ requires m/z

423.3626), 405.3526 ($C_{30}H_{45}$ requires m/z 405.3521), 299.2379 ($C_{21}H_{31}O$ requires m/z 299.2374), and 283.2640 ($C_{18}H_{35}O_2$ requires m/z 283.2636); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 5.52 (1H, dd, J = 8.0, 3.2 Hz, H-15), 4.50 (1H, dd, J = 10.1, 6.2 Hz, H-3α), 3.09 (1H, dd, J = 5.0, 4.6 Hz, H-11β), 2.78 (1H, d, J = 4.6 Hz, H-12β), 2.28 (2H, t, J = 7.4 Hz, H-2′), 1.60 (m, H-3′), 1.23 (chain, 28H), 1.08, 1.06, 0.98, 0.94, 0.88 (3H each, s, 5 x CH_3), 0.87 (3H, t, J = 7.4 Hz, H-18′), 0.86, 0.84, and 0.80 (3H each, s, 3 x CH_3); ^{13}C-NMR: See Table 1; HMBC: See Figure 2.

Table 1. ^{13}C-NMR Spectral Data of Crassifoate (**2**) and 11α, 12α-Oxidotaraxerol (**2a**).

Carbon	(2)	(2a)	Carbon	(2)	(2a)
1	38.0	38.2	19	40.3	40.3
2	23.3	26.9	20	28.7	28.7
3	80.3	79.0	21	36.6	36.6
4	37.7	38.7	22	38.2	38.2
5	54.7	54.6	23	27.9	27.9
6	18.8	18.9	24	17.0	17.0
7	33.2	33.2	25	16.6	15.4
8	38.9	38.9	26	27.0	27.1
9	51.9	52.0	27	30.2	30.2
10	37.6	37.5	28	29.9	29.9
11	53.5	53.6	29	33.7	33.7
12	58.2	58.3	30	19.5	19.6
13	36.6	36.6	1	173.5	–
14	157.1	157.1	2	34.8	–
15	118.9	118.9	3	25.2	–
16	35.3	35.2	17	22.7	–
17	35.4	35.4	18	14.1	–
18	48.1	48.1	Chain	29.2-29.7	–

In $CDCl_3$ at 100 MHz.

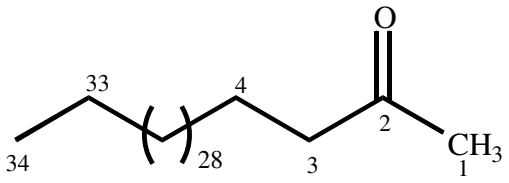
Fraction eluted with 30% chloroform in hexane yielded **3** as a mobile oil (0.7 mg).

Crassifol (3)

$[\alpha]_D^{28}$ = +72.3 ° (chloroform, c 0.778); IR ($CHCl_3$): 3425 (OH), 1720 (ketone C=O) cm^{-1}; EIMS: m/z 312 $[M]^+$, 294 $[M\text{-}H_2O]^+$, 269 $[M\text{-}COCH_3]^+$, and 251 $[M\text{-}COCH_3 + H_2O]^+$; HR-EIMS: m/z 312.3032 ($C_{20}H_{40}O_2$ requires m/z 312.3028), 294.2926 ($C_{20}H_{38}O$ requires m/z 294.2922), 269.2849 ($C_{18}H_{37}O$ requires m/z 269.2844), and 251.2743 ($C_{18}H_{35}$ requires m/z 251.2738); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 2.38 (2H, t, J = 7.5 Hz, H-4), 2.11 (3H, s, H-1), 1.51 (3H, s, H-3a), 0.86 (6H, d, J = 6.6 Hz, H-15a & 16), 0.85 (3H, d, J = 6.9 Hz, H-7a), and 0.83 (3H, d, J = 6.9 Hz, H-11a); ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 29.8 (C-1), 209.3 (C-2), 77.2 (C-3), 29.7 (C-3a), 44.1 (C-4), 21.4 (C-5), 36.5 (C-6), 32.7 (C-7), 19.5 (C-7a), 37.4 (C-8), 24.4 (C-9), 37.2 (C-10), 32.8 (C-11), 19.7 (C-11a), 37.3 (C-12), 24.8 (C-13), 39.4 (C-14), 28.0 (C-15), 22.6, and 22.7 (C-15a & 16); HMBC: See Figure 3.

Results and Discussion

The ethanol soluble part of *Nepeta crassifolia* collected from Kangavar, Iran, yielded a long chain ketone (**1**, crassifone), a fatty acid ester of triterpene (**2**, crassifoate), and a diterpene (**3**, crassifol).



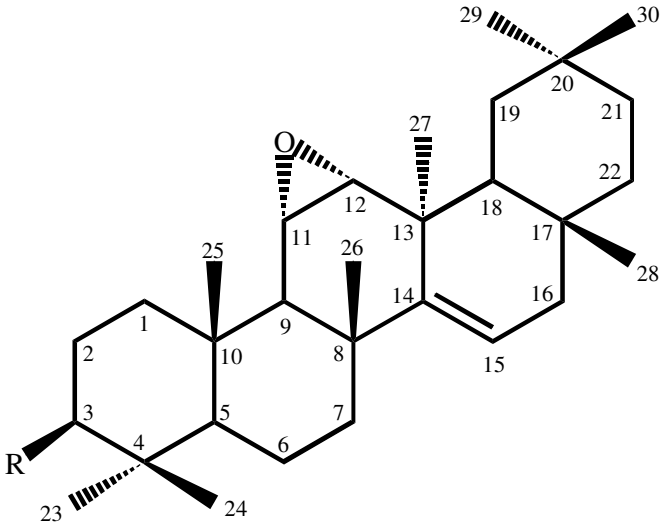
Crassifone (**1**)

Crassifone (1)

Compound **1** was obtained as a white solid. The IR spectrum of **1** displayed a strong absorption band at 1720 cm^{-1} due to the presence of ketonic function in the molecule. The molecular mass, 492 a.m.u., was depicted *via* EIMS corresponding to the formula $C_{34}H_{68}O$ determined through HREIMS. The proton NMR spectrum of **1** displayed 2 methyl signals: a singlet at δ 2.11 and a triplet at δ 0.86 (J = 6.9 Hz) due to Me-1 and Me-34, respectively. Their corresponding carbons resonated at δ 29.8 (Me-1) and 14.1 (Me-34).

The protons and their associated carbon signals were correlated with the aid of HMQC experiments. Finally, the structure was deduced through HMBC experiments (Figure 1) as **1** and named crassifone. The described new long chain ketone is a new addition to the list of natural products.

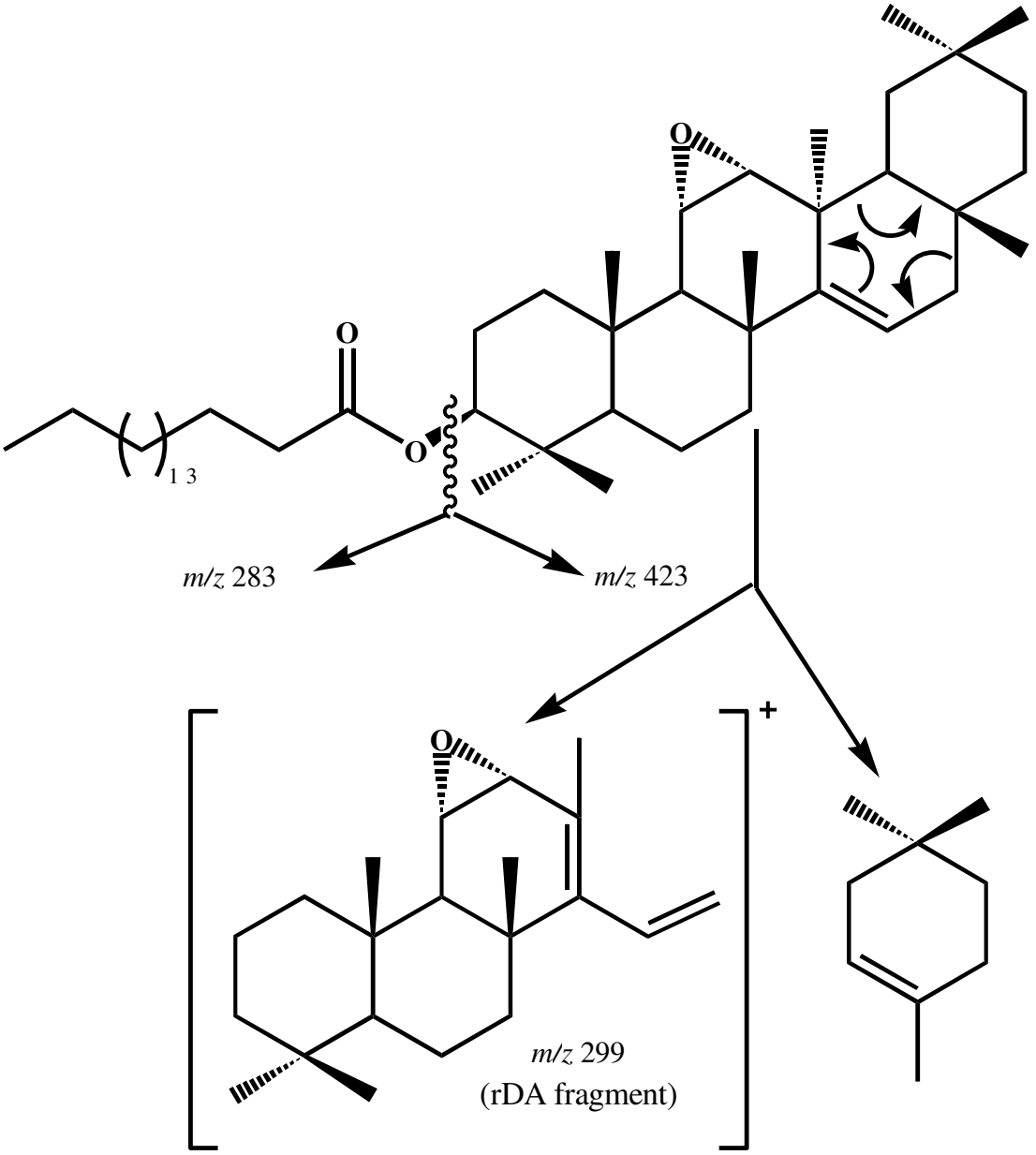
Figure 1. Important HMBC interactions in **1**.



(**2**) R = stearyl (crassifoate)
(**2a**) R = OH

Crassifoate (2)

The molecular weight and formula of compound **2** were determined by EI-MS and HR-EI-MS as 706 a.m.u. and $C_{48}H_{82}O_3$. The fragments at m/z 423 and 283 in the EIMS were due to the loss of stearyl moiety from the molecular ion peak and stearyl fragment, respectively, and their corresponding formulae were determined through HR-EI-MS. The peak at m/z 299 was due to the retro Diels-Alder fragment confirming the position of a double bond in **2** (Scheme). The other mass spectral details are given in the experimental section. The IR spectrum of **2** exhibited absorptions at 1735, 1640, and 1190 cm^{-1} due to the ester carbonyl, olefinic, and oxirane functionalities in the molecule.



Scheme. Retro Diels-Alder (rDA) fragmentation in crassifoate (**2**)

The ^{1}H-NMR spectrum of **2** showed 2 downfield double doublets at δ 5.52 (J = 8.0, 3.2 Hz) and 4.50 (J = 10.1, 6.2 Hz) assigned to olefinic (H-15) and H-3α protons. The β-protons due to oxirane function appeared at δ 3.09 (dd, J = 5.0, 4.6 Hz, H-11) and at δ 2.78 (d, J = 4.6 Hz, H-12). In all, the same spectrum displayed 9 methyl signals due to 8 quaternary methyls and a primary methyl. The 8 singlets between δ 0.88-1.08 and 0.80-0.86 were due to the triterpenic portion of the molecule, while the triplet at δ 0.87 (J = 7.4 Hz) was due to the stearyl moiety (H-18') coupled with the triterpene part at C-3. The protons associated with the carbon chain of the stearyl part appeared as a broad singlet at δ 1.23.

The ^{13}C-NMR spectrum of **2** exhibited 2 quaternary downfield signals at δ 173.5 and 157.1 due to the ester carbonyl and olefinic quaternary carbon, respectively. The signal of olefinic methine was observed at δ 118.9, and the signals associated with the oxirane-ring appeared at δ 53.5 (C-11) and 58.2 (C-12) in the B.B./DEPT spectra. The signal due to C-3 resonated at δ 80.3. Details of the remaining carbon signals are given in the Table 1.

The signals in the ^{13}C-NMR spectra (B.B. and DEPT) of compound **2**, when compared to the 3β-hydroxy-11α, 12α-epoxy-friedoolean-14-ene (**2a**) (Table 1)[16] and conclusions, were cross checked with HMBC connectivities (Figure 2), the structure of **2** was elucidated as 3β-hydroxy-11α, 12α-epoxy-friedoolean-14-en-3β-stearate and named crassifoate. This fatty acid ester of triterpene is also a new addition to the natural products. These types of secondary metabolites are not very common and are rarely reported in the literature.[17]

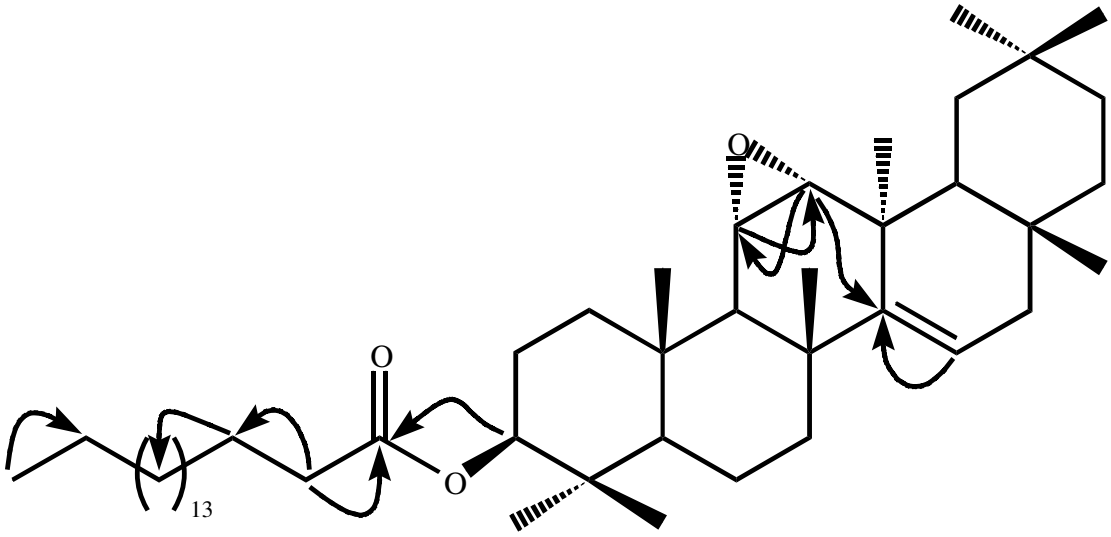
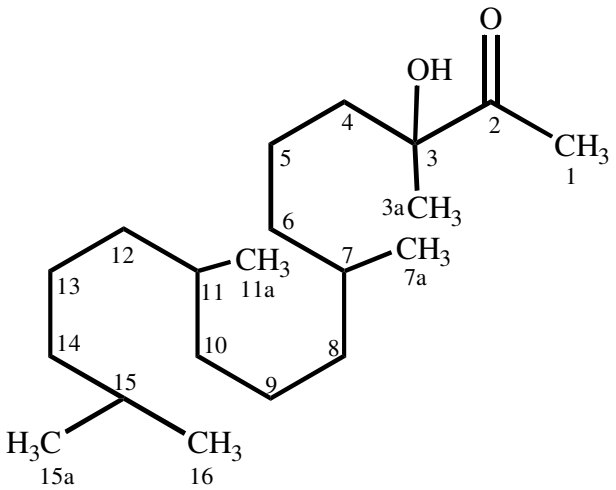


Figure 2. Important HMBC interactions in **2**.

Crassifol (3)

The third compound (**3**) was obtained as a light mobile oil. The IR spectrum of **3** displayed strong absorption bands at 3425 and 1720 cm^{-1} due to the presence of hydroxyl and ketone carbonyl functionalities, respectively. The molecular mass and corresponding formula were determined based on electron impact (EI) and high resolution electron impact (HR-EI) mass spectra at m/z 312 $(M)^+$ corresponding to $C_{20}H_{40}O_2$. The fragment due to the removal of a water molecule from the molecular ion was observed at m/z 294. Two

more fragments at m/z 269 and 251 were identified in the spectrum due to the loss of acetyl and acetyl plus water from the molecular ion, respectively.



Crassifol (**3**)

The proton NMR spectrum of **3** displayed 2 doublets of 3H at δ 0.83 and 0.85 with the coupling constants of 6.9 Hz due to the methyls situated at C-11 and C-7, while a 6-proton doublet at δ 0.86 ($J = 6.6$ Hz) was due to Me-16 and 15a. The 2 singlets at δ 1.51 and 2.11 were due to Me-3a and Me-1, respectively.

The carbon-signals due to the methyls were located in the broad-band (B.B.) and DEPT spectra at δ 29.8 (Me-1), 29.7 (Me-3a), 19.5 (Me-7a), 19.7 (Me-11a), and 22.6-22.7 (Me-15a and 16), and were cross-checked through HMQC experiments. The B.B. spectrum displayed only 2 quaternary-carbon signals at δ 77.2 and 209.3 assigned to carbinylic (C-3) and ketonic (C-2) carbons.

The obtained information when gathered with HMBC connectivities (Figure 3), the structure of

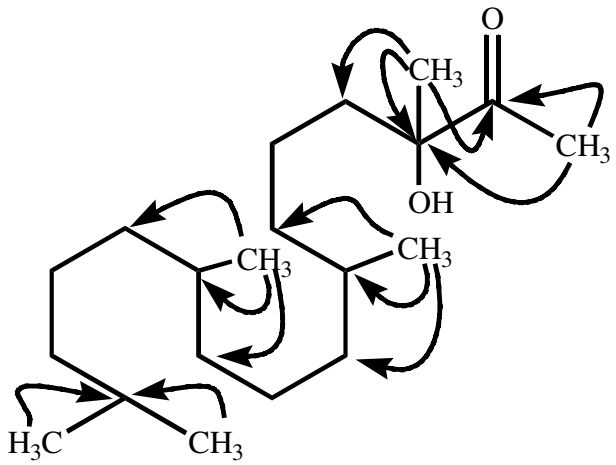


Figure 3. Important HMBC interactions in **3**.

discussed compound was concluded to be **3** and named crassifol, having a phytane type skeleton. The quaternary methyl (H-3a) at δ 1.51 gives HMBC connectivity with carbonyl carbon (δ 209.3) as well as

quaternary carbon at δ 77.2 (C-3), rules out the possibility of hydroxyl function at any carbon, except C-3. Such acyclic diterpenoids are reported in the literature.[15] To date, crassifol has not been reported from any natural source.

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References

1. A.I. Pojarkova, "**Flora of the U.S.S.R. Moscow"**, Izdatel'stvo Akademii Nauk SSSR, Vol. XX, pp. 191-293, 1954.
2. I.C. Hedge, **Proceedings of the Royal Society of Edinburgh**, **89B**, 23-25 (1986).
3. M. Dabiri and F. Sefidkon, **Flav. Fragr. J.**, **18**, 225-227 (2003).
4. Avicenna, "**Canon of Medicine**", Nizami Press, Kanpur, 1906.
5. Avicenna, "**Kitabul Adviyatul Qalbia**", National Printing Co, Aligarh, 1956.
6. A.W. Sherani, "**Tazkira Imam Sawedi**", Illahiya Press, Egypt, 1887.
7. G.R. Amin, "**Popular Medicinal Plants of Iran**, **Vol. 1**". Ministry of Health Publications, Tehran, pp. 40-44, 1991.
8. R.N. Chopra, S.I. Nayar and I.C. Chopra, "**Glossary of Indian Medicinal Plants**", CSIR New Delhi, pp. 174-175, 1956.
9. G.A. Stuart, "**Chinese Materia Medica**", Shanghai, pp. 281, 1911.
10. K.M. Nadkarni, **Indian Materia Medica**, **1**, 845-846 (1976).
11. S.D. Sastry, W.R. Springstube and G.R. Waller, **Phytochemistry**, **11**, 453-455 (1972).
12. J.G. Urones, I.S. Marcos, P.B. Barcala and A.M.L. Bertelloni, **Phytochemistry**, **27**, 1525-1527 (1988).
13. S.P.S. Bhandari, H.S. Garg, P.K. Agrawal and D.S. Bhakuni, **Phytochemistry**, **29**, 3956-3958 (1990).
14. V.U. Ahmed, M. Noorwala, F.V. Mohammad and M.G. Shah, **Planta Med.**, **59**, 366-368 (1993).
15. V.U. Ahmed and M.S. Ali, **Phytochemistry**, **30**, 4172-4174 (1991).
16. K. Usmanghani, S. Bano, G.H. Rizwani, M. Ahmed and V.U. Ahmed, **Fitoterepia**, **60**, 351-352 (1989).
17. M.L. Barreiros, J.M. David, P.A. Pereira, M.L.S. Guedes and J.P. David, **J. Braz. Chem. Soc.**, **13**, 669-673 (2002).