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THE ISOLATION AND TOTAL SYNTHESIS OF CADABICINE METHYL ETHER

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A new spermidine alkaloid, cadabicine methyl ether (1) has been isolated from the stem bark of two capparidacious plants, *Cadaba farinosa*, Forssk., and *Crataeva nurvala* Bunch- Ham, using the same isolation procedure. The structure of (1) was determined by spectroscopic studies of the alkaloid and its N-acetyl derivative and confirmed by its total synthesis.

Key words: *Cadaba farinosa*, Alkaloid, Isolation, Synthesis.

Introduction

Cadaba farinosa, Forssk. (Syn. *C. fruticosa*, *L. Druce*, *C. indica*, Lam.) and *Crataeva nuvala*, Buch-Ham. (syn. *C. adansonii*, *C. religiosa* Hook.), belonging to the Capparidaceae (Cappariaceae) family, are commonly found in Pakistan. *C. nurvala* is also cultivated in southern parts of Pakistan. The different parts of these plants have many medicinal properties [1,2]. From *Cadaba farinosa*, the isolation of stachydrine [3] from the leaves, a dilactone, cadabalone [4], from the pods, two alkaloids, cadabicine [5] and cadabicine diacetate [6] and a sesquiterpene cadabicilone [7] from the stem bark of the plant have been reported. The reported constituents of *Crataeva nurvala* are different triterpines and salts, and also cadabicine and cadabicine diacetate [6]. In the present communication we wish to report the isolation, structure elucidation and total synthesis of a new spermidine alkaloid, cadabicine methyl ether (1), from the stem barks of these plants. In isolation process we did not use methanol in presence of a base therefore it is not a based catalysed methylation production of cadabicine.

Experimental

Melting points were recorded in glass capillary tubes on Gallenkamp melting point apparatus and are uncorrected. The UV (MeOH) spectra were recorded on Pye Unicam SP-800 A spectrometer. IR (KBr) spectra were recorded on JASCO IRA-1 and JASCO A-302 infrared spectrometer. The ^{1}H n.m.r. and ^{13}C n.m.r. spectra were recorded at Bruker AM-300 spectrometer, operating at 300 MHz and 75.4 MHz respectively. The MS spectra were recorded on varian MAT- 112 and MAT-113 spectrometers connected to PDP 11/34 computer system. The reagents and solvents were obtained from E. Merck and Fluka.

Extraction of plant material. Stem barks of *Cadaba farinosa* and *Crataeva nurvala* (31 kg each) were collected from Karachi and chopped into small pieces. The coarsly powdered form of the material was soaked in EtOH for 15 days at room temperature. The extract was obtained after evaporation of ethanol. The ethanolic extractive was defatted by partitioning it between H_2O and EtOAc. The aqueous layer was basified with NH_3 (pH = 9) and extracted repeatedly with $CHCl_3$. The evaporation of $CHCl_3$ layer yielded a brown gummy crude alkaloidal material. The total weight of crude alkaloids was 11.4 g in case of *Cadaba farinosa* and 6.6 g in case of *Crataeva nurvala*.

The crude alkaloidal mixture was chromatographed by flash column and eluted with CH_2Cl_2 with increasing polarity of MeOH. The crude alkaloidal mixture was partially separated into two portions, the faster moving and slower moving alkaloidal mixture. The faster moving mixture contains some organic basis of low melting points whose structures are undetermined.

The separated slower moving alkaloidal mixture was subjected to repeated silica gel column chromatography using CH_2Cl_2, MeOH, NH_3 (80:18:2), as mobile phase, which furnished cadabicine methyl ether (250 mg) alongwith small amount of cadabicine (40 mg). It was purified through repetitive short column chromatography on silica gel, using CH_2Cl_2, MeOH, NH_3 (82:17:1) as eluant. Fractional crystallization from MeOH as well as acetone-water (1:1) yielded light brown crystals (55 mg) of (1), m.p. 190-92°. The purity of compound (1) was confirmed by h.p.t.l.c., as well as on h.p.l.c. (Z-Module, RP-18 cartridge) with MeOH-water (70:30) as mobile phase.

Cadabicine methyl ether (1). λmax: 220 (log ε = 2.84), 285 (log ε = 2.78) and a shoulder at 310 nm. υmax: 3400 (NH), 1660 (α, β- unsat. amide) and 1600 cm^{-1} (aromatic ring). For ^{1}H n.m.r. see text, for ^{13}C n.m.r. see Table 1, and for high resol. Scheme 1.

Cadabicine methyl ether acetate (2).- Compound (1) was dissolved in Ac_2O-C_5H_5N (1.5 : 0.5 ml) with warming and then kept overnight at ambient temperature. On addition of water, the N- acetate was obtained as an amorphous solid. It was recrystallised from hot MeOH. The colourless crystals melted at 180-82°. λmax 219 (log ε =2.88), 284 (log ε = 2.74) and a

shoulder at 318 nm. υmax 3400 (NH), 1740 (N-$COCH_3$), 1655 (α, β-unsat. amide) and 1595 cm^{-1} (aromatic ring). δ(DMSO-d_6) 1.88-1.89 (s, 3H, $NCOCH_3$), 1.21-1.49 (m, 6H, 3 x CH_2, attached to other methylene groups), 3.15-3.39 (m, 8H, 4 x CH_2 attached to nitrogen atoms), 3.73 (s, 3H, OCH_3), 5.75, 6.56, 7.30, 7.55 (each d, 1H, J = 15.6 Hz, olefinic protons of trans-cinnamic acid), 6.55 (d, 1H, J = 3.5 Hz, H-27), 6.82 (dd, 1H, J = 3.1, 8.2 Hz, H-24), 7.19 (d, 1H, J = 8.2 Hz, H-25), 7.12, 7.61 (each d, J = 8.3 Hz, para-disubstitute benzene ring). For ^{13}C n.m.r. Table 2. High resol. MS: *m/z* found (calcd. for), 491.2421 ($C_{28}H_{33}N_3O_5$, 491.2420) [M^+], 449.2251 ($C_{26}H_{31}N_3O_4$, 449.2314) [M^+ - $COCH_2$], 435.2151 ($C_{25}H_{29}N_3O_4$, 435.2157) [M^+-$COCH_2$- CH_2]$^+$, 419.2210 ($C_{25}H_{29}N_3O_3$, 419.2208), [M^+ - $COCH_2$-CH_2O]$^+$, 390.1941 ($C_{24}H_{26}N_2O_5$, 390.1943), 348.1589 ($C_{22}H_{22}NO_3$, 348.1599), 321.1010 ($C_{19}H_{15}NO_4$, 321.1001), 305.0814 ($C_{19}H_{13}O_4$, 305.0813), 276.0788 ($C_{18}H_{12}O_3$, 276.0786), 249.0914 ($C_{17}H_{13}O_2$, 249.0915), 231.1262 ($C_{14}H_{17}NO_2$, 231.1259), 189.1155 ($C_{12}H_{15}NO$, 189.1153), 160.0526 ($C_{10}H_8O_2$, 160.0524), 131.0497 (C_9H_7O, 131.0496).

Diphenyl ether (5). 1.8503 g 4-bromobenzaldehyde (3), 1.5215 g Isovanilline (4) and 0.715 g Cu_2O are allowed to react in boiling N, N-dimethyl acetamide for about 20 hrs, under nitrogen atmosphere. The prepared compound was separated by extraction with ether and purified on a silica gel column. The amount of compound (5) obtained was 1.782 g. υmax: 1700 cm^{-1} (Two overlapped bands for 2 x CHO groups). δ($CDCl_3$) 8.9 (1H, CHO) and δ9.1 (1H, CHO), HRMS: *m/z* 256.0756 (calcd. for $C_{15}H_{12}O_4$, 256.0735), 227.0706 (M^+-CHO), 198.0681 (M^+-2 x CHO).

Diester (6). 1.28 g of compound (5) was treated with phosphonium yield of α-bromo methyl acetate (1:2 moles) in benzene. The resulting diester (6) was extracted by CH_2Cl_2 from the mixture. υmax: 1720 cm^{-1} and 1725 cm^{-1} (Two ester groups). δ($CDCl_3$) 3.75 and 3.77 (2 x $COOCH_3$), 3.82 (O-CH_3) 6.24, 6.32, 7.52, 7.63 (each d, 1H, J = 15.6 Hz, olefinic protons *of trans*-cinnamic acid), 7.31, 7.63 (each d, J = 9.0 Hz, *para* disubstituted benzene ring), 6.91 (d, 1H, J = 3.1 Hz, H-27), 7.0 (d, 1H, 9.0 Hz, H-25) and 7.21 (dd, 1H, J = 3.2 and 9.0 Hz), 7.31, 7.63 (each d, J = 9.0 Hz, *para* disubstituted benzene ring). HRMS: *m/z* 368.1262 (calcd. for $C_{21}H_{20}O_6$, 368.1259), [M^+], 337.1073 (calcd. for $C_{20}H_{17}O$, 337.1075) [M^+-OCH_3], 221.0972 (calcd. for $C_{16}H_{13}O$, 221.0965) [M^+- OCH_3-2 x $COOCH_2$].

Diacid (7). The alkaline hydrolysis of diester yields a dicarboxylic acid which is extracted by hot benzene, on evaporation a yellowish white crystalline compound is obtained in overall 81% yield from compound (5). υmax: 1695 and 1700 cm^{-1} (two COOH groups). δ($CDCl_3$) 9.97 (a sharp singlet of 2H for 2 x COOH), other peaks are almost similar to compound (6). Molecular ion peak was not observed and other peaks are similar to compound (6).

Thiazolidine compound (8). 340 mg of compound (7) on treatment with thionyl chloride formed a thick gummy acid chloride in liquid form. This oily acid chloride was treated with Thallium (I) salt [12] of thiazolidine-2-thione with continuous stirring in THF at room temperature under nitrogen atmosphere for about 20 hrs. A dirty white solid (8) was obtained by extraction of mixture with CH_2Cl_2. It was recrystallized from $CHCl_3$. The compound (8) is formed in 48% yield (260 mg). υmax: 1680 cm^{-1} (C=O), δ($CDCl_3$) 3.37, 3.56, 3.98, 3.68 (each t, J = 7.00 Hz, four methylenes of thiazolidine moiety), 3.65 (s, OCH_3), 7.44, 7.56, 7.62, 7.71 (each d, 1H, J = 15.6 Hz, olefinic protons of *trans*- cinnamic acid), 6.93 (d, 1H, J = 3.0 Hz, H-27), 7.02 (d, 1H, 9.0 Hz, H-25), 7.26 (dd, 1H, J = 3.1 and 9.0 Hz, H-24) 7.33, 7.78 (each d, J=9.0 Hz, *para* disubstituted benzene ring). HRMS: *m/z* 542.0466 (calcd. for $C_{25}H_{22}O_4N_2S_4$, 542.0462), [M^+], 308.1046 (calcd. for $C_{19}H_{16}O_4$, 308.1048), [M^+-2 x thiazolidine moieties).

Compound (1) from thiazolidine compound (8).- 200 mg of compound (8) were allowed to react with spermidine by the known method [12]. The crude product was purified through h.p.t.l.c. (E. Merck Cat. No. 5629) using $CHCl_3$:MeOH: H_2O:NH_3 (7 : 2 : 1.5 : 0.5) as developing solvent. The pure cadabicine methyl ether was obtained in 34% yield (56.3 mg).

N-acetyl cadabicine methyl ester (2) from synthetic compound (1).- Acetyl derivative of compound (1) was prepared by the same procedure as for compound (2) from isolated cadabicine methyl ether (1).

Results and Discussion

The crude alkaloidal material from the stem bark was partitioned between H_2O and CH_2Cl_2. The residue from CH_2Cl_2 layer was separated by silica gel column chromatography, which led to the isolation of pure cadabicine methyl ether, m.p. 190-92°. The high resolution mass spectrum, showed a molecular ion peak at *m/z* 449.2282 corresponding to molecular formula $C_{26}H_{31}N_3N_4$ (calcd. 449.2314). The UV spectrum showed maxima at 220 (logε = 2.84), 282 (logε = 2.78) and a shoulder at 310 nm. These values are very close to those of cadabicine [5] and codonocarpine [8].

The IR spectrum has bands at 3400-3200 (br. NH), 1660 (α, β-unsat. amide) and 1600 cm^{-1} (aromatic ring).

The molecular formula shows that the compound has one "CH_2" unit higher than that of cadabicine ($C_{25}H_{29}N_3O_4$). The peak at δ3.56 in ^1H n.m.r. spectrum and at δ56.22 in ^{13}C n.m.r., show the presence of a methoxy group and negative test for phenol with $FeCl_3$ reagent indicates that the new isolated compound may be a methyl ether of cadabicine.

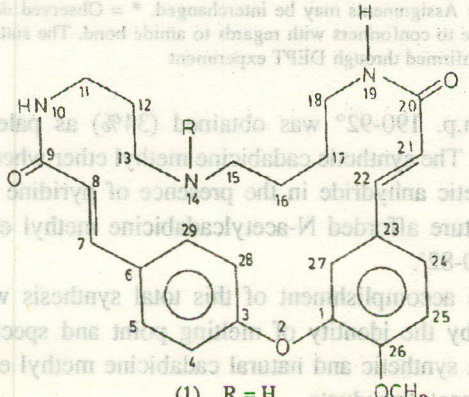
The ^1H n.m.r. and ^{13}C n.m.r. Table 1 spectra of cadabicine methyl ether showed doubling of several signals, which is due to the slowly interconverting *E* and *Z* conformers with regard

to amide bonds, in solution. The same phenomenon has been reported earlier in amides, [9,10] and also observed by us in cadabicine [5]. The ^{1}H n.m.r. (DMSO-d_6) spectrum of (1) showed two multiplets appearing between δ1.22-1.71 (6H) and δ3.15-3.39 (8H) which are assigned to three methylene groups adjacent to other methylenes and four methylene groups adjacent to electronegative nitrogen atoms respectively. A sharp singlet at δ3.56 proved the presence of a methoxy group. The four doublets at δ5.83, 6.59, 7.19 and 7.48 (each 1H, J = 15.5 Hz), indicate the presence of four olefinic protons of the two *trans*-cinnamic acid moieties. A doublet at δ6.92 (J=3.1 Hz) with meta coupling only is assigned to H-27. The two doublets at 7.19 and 7.84 (each 2H, J = 8.2 Hz), with *ortho* coupling only, are typical for a *para* disubstituted benzene ring. A doublets at δ6.97 (1H, J = 8.2 Hz) with *ortho* coupling only is attributed to H-25. The H-24 gives a doublet at δ7.02 (1H, J = 8.2 ad 3.0 Hz) with *ortho* and *meta* couplings. The assignments have been confirmed through 2D-J-resolved experiments.

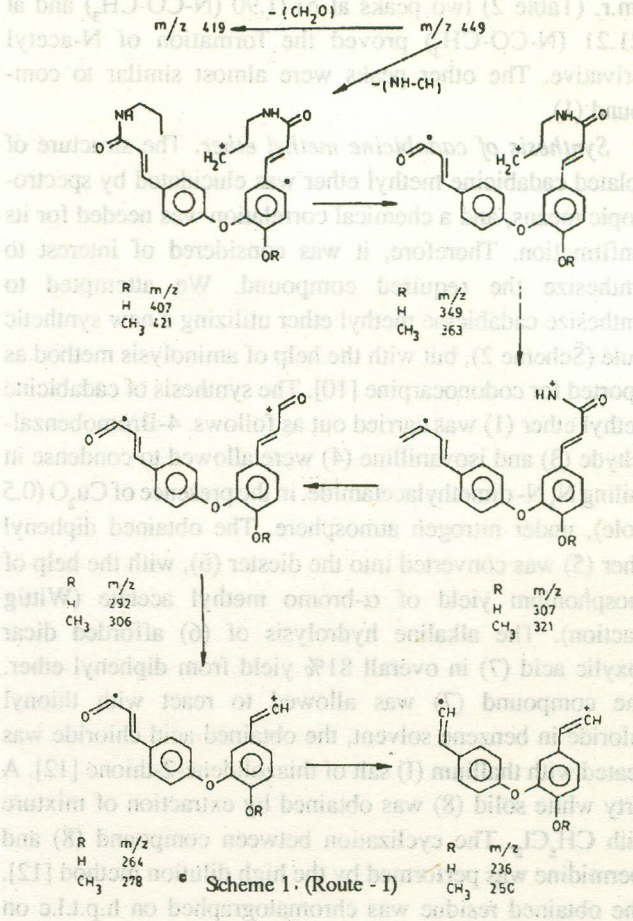
The spermidine moiety in (1) may be joined with the rest of the molecule in two different manners, but the mass fragmentation (Scheme 1) and its comparison with that of cadabicine, whose structure has been proved through X-ray crystallography [5], proves that the spermidine is joined in the manner shown in structure (1) and the alternative structure with opposite attachment of the spermidine can be ruled out.

Cadabicine methyl ether was dissolved in pyridine and allowed to react with acetic anhydride at normal temperature. On addition of cold water, the N-acetyl derivative (2) was obtained as amorphous solid. It was recrystallised from hot MeOH. The colourless crystals melted at 180-82°. The HRMS of N-acetate gave a peak at *m/z* 491.2421 attributable to the molecular formula $C_{28}H_{33}N_3O_5$ (calcd. 491.2420). The other important fragments were at *m/z* 449 [M^+ -CH_2-C=O]$^+$, 435, 419, 390, 348, 321, 305, 276, 249, 231, 189 and 160.

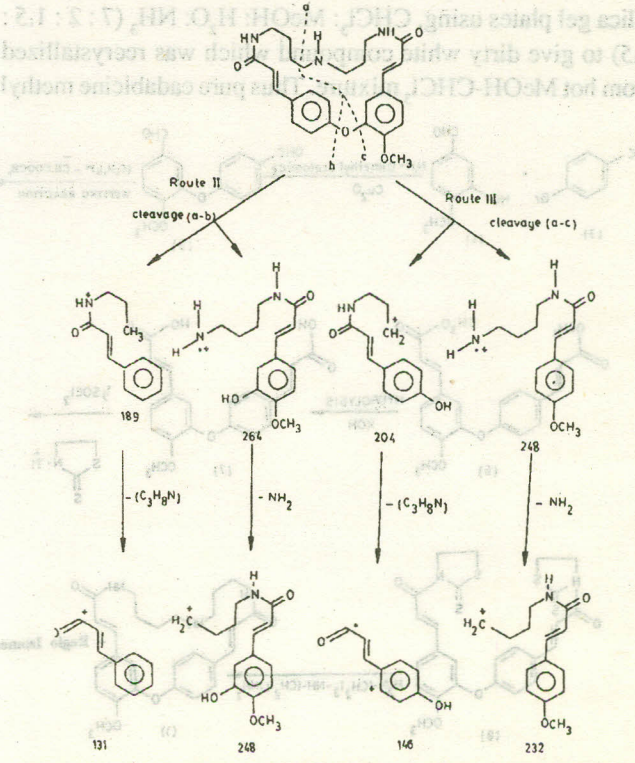
In the IR (KBr) spectrum, a band at 1740 cm^{-1} (N-$COCH_3$), in ^{1}H n.m.r. a peak at δ1.9 (N-CO-CH_3) and in ^{13}C



(1) R = H,
(2) R = Ac,



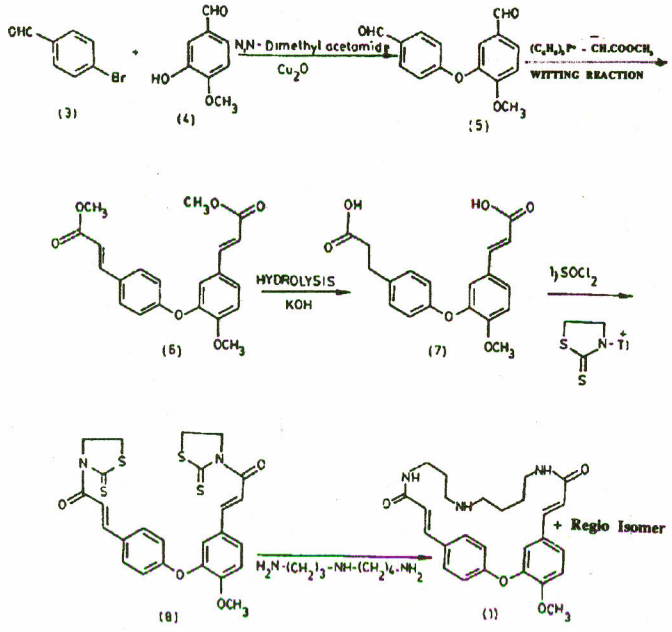
Scheme 1 · (Route - I)



Scheme 1 · (Route - II and III)

n.m.r. (Table 2) two peaks at δ171.90 (N-CO-CH_3) and at δ21.21 (N-CO-CH_3) proved the formation of N-acetyl derivative. The other peaks were almost similar to compound (1).

Synthesis of cadabicine methyl ether. The structure of isolated cadabicine methyl ether was elucidated by spectroscopic means, and a chemical correlation was needed for its confirmation. Therefore, it was considered of interest to synthesize the required compound. We attempted to synthesize cadabicine methyl ether utilizing a new synthetic route (Scheme 2), but with the help of aminolysis method as reported for codonocarpine [10]. The synthesis of cadabicine methyl ether (1) was carried out as follows. 4-Bromobenzaldehyde (3) and isovanilline (4) were allowed to condense in boiling N, N- dimethylacetamide, in the presence of Cu_2O (0.5 mole), under nitrogen atmosphere. The obtained diphenyl ether (5) was converted into the diester (6), with the help of phosphonium yield of α-bromo methyl acetate (Wittig reaction). The alkaline hydrolysis of (6) afforded dicar boxylic acid (7) in overall 81% yield from diphenyl ether. The compound (7) was allowed to react with thionyl chloride in benzene solvent, the obtained acid chloride was treated with thallium (I) salt of thiazolidene-2-thione [12]. A dirty white solid (8) was obtained by extraction of mixture with CH_2Cl_2. The cyclization between compound (8) and spermidine was performed by the high dilution method [12]. The obtained residue was chromatographed on h.p.t.l.c on silica gel plates using, $CHCl_3$: MeOH: H_2O: NH_3 (7 : 2 : 1.5 : 0.5) to give dirty white compound which was recrystallized from hot MeOH-$CHCl_3$ mixture. Thus pure cadabicine methyl



(Scheme 2)

TABLE 1. ^{13}C-NMR CHEMICAL SHIFTS OF CADABICINE METHYL ETHER (1) (DMSO-d_6, 100 MHz).

Carbon	ppm	Carbon	ppm
1	148.71	17	25.63
3	155.41	18	43.93/44.24*[d]
4	123.43	20	164.94/165.08*[c]
5	129.26	21	125.43/125.77*[b]
6	133.72	22	137.79/138.14*[a]
7	137.54/137.62*[a]	23	128.88
8	125.82/125.97*[b]	24	121.76
9	164.61[c]	25	112.08
11	45.05/46.16*[d]	26	140.12
12	25.96/26.13*[c]	27	118.94
13	38.63/39.40*	28	120.26
15	35.28/35.95	29	129.33/129.45*
16	22.29/22.66*	OCH_3	56.22

a,b,c,d,e = Assignments may be interchanged. * = observed doubling of signals due to conformers with regards to amide bond. The status of each carbon confirmed through DEPT experiment

TABLE 2. ^{13}C-NMR CHEMICAL SHIFTS FOR N-ACETYL CADABICINE METHYL ETHER (2) (DMSO-d_6, 100 MHz).

Carbon	ppm	Carbon	ppm
1	149.16	18	45.07/45.62*[d]
3	153.28	20	168.24/169.13*[c]
4	122.44	21	126.24/126.44*[b]
5	122.23	22	140.13/140.32*[a]
6	130.14	23	128.34
7	140.38/140.49*[a]	24	122.84
8	125.76/125.89*[b]	25	114.08
9	166.62[c]	26	147.34
11	46.08/46.22*[d]	27	118.70
12	27.74/27.88*[e]	28	125.85
13	37.18/38.16*	29	129.28/129.46*
15	35.62/35.74*	OCH_3	52.82
16	23.45/23.50*	$NOCCH_3$	170.84
17	26.85[e]	$NCOCH_3$	21.82

a,b,c,d,e = Assignments may be interchanged. * = Observed doubling of signals due to conformers with regards to amide bond. The status of each carbon confirmed through DEPT experiment

ether, m.p. 190-92° was obtained (34%) as pale yellow crystals. The synthetic cadabicine methyl ether when treated with acetic anhydride in the presence of pyridine at room temperature afforded N-acetylcadabicine methyl ether (2), m.p. 180-82°.

The accomplishment of this total synthesis was confirmed by the identity of melting point and spectral data between synthetic and natural cadabicine methyl ether and their N-acetyl products.

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