New Tropane Alkaloids from *Erythroxylum moonii*

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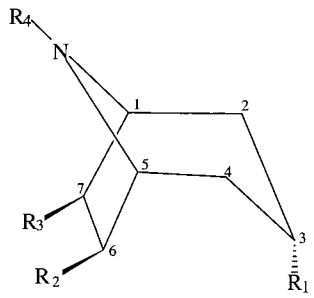
Four new tropane alkaloids were isolated from the leaves of *Erythroxylum moonii* and identified as 3α-isobutyryloxy-7*β*-hydroxynortropane (**1**), 3α-hydroxy-7*β*-phenylacetoxynortropane (**2**), 3α-*cis*-cinnamoyloxytropane (**3**), and 3α-hydroxy-6*β*-(3′-hydroxy-2′-methyl-3′-phenylpropionyloxy)-7*β*-hydroxytropane (**4**). Other alkaloids isolated for the first time from *E. moonii* were 3α-benzoyloxytropane, 3α-phenylacetoxytropane, 3α-*trans*-cinnamoyloxytropane, and 3α-phenylacetoxy-6*β*,7*β*-dihydroxynortropane. The structures of compounds **1**−**4** were elucidated by spectroscopic methods.

Tropane alkaloids are an important class of natural products because of their analgesic, anesthetic, anticholinergic, antiemetic, antihypertensive, parasympatholytic, and many other pharmacological actions.[1,2] In modern medicine, three tropane alkaloids, atropine, hyoscyamine, and scopolamine, are among the major drugs of plant origin. Cocaine isolated from *Erythroxylum coca* is another widely used and important natural product bearing a tropane ring system.[3] Although synthetic approaches have been developed for the basic tropane alkaloids, most of the pharmaceutically important alkaloids of this series are still obtained from plant sources. The family Erythroxylaceae has proved to be a rich source of tropane alkaloids.[4] *Erythroxylum moonii* Hoehr. is a small shrub which is used in Sri Lanka as an effective antihelmintic for roundworms and for the suppuration of boils and abscesses.[5] In preliminary work, an alcoholic extract of *E. moonii* showed antifungal activity against certain pathogenic fungi and led to the isolation of new dimeric tropane alkaloids.[6] We report here the isolation of four new monomeric tropane alkaloids (**1**−**4**, Figure 1) from this plant.

The alkaloids obtained from the alcoholic extract of the leaves of the plant were purified by column and thin-layer chromatography. Their structures were elucidated by the application of spectroscopic techniques.[1,7] They comprise the new alkaloids 3α-isobutyryloxy-7*β*-hydroxynortropane (**1**), 3α-hydroxy-7*β*-phenylacetoxynortropane (**2**), 3α-*cis*-cinnamoyloxytropane (**3**), and 3α-hydroxy-6*β*-(3′-hydroxy-2′-methyl-3′-phenylpropionyloxy)-7*β*-hydroxytropane (**4**), along with the known bases 3α-benzoyloxytropane,[8] 3α-phenylacetoxytropane,[7,9] 3α-*trans*-cinnamoyloxytropane,[10,11] and 3α-phenylacetoxy-6*β*,7*β*-dihydroxynortropane.[12] All these known compounds were isolated for the first time from the leaves of *E. moonii*.

The HREIMS of the new alkaloid (3α-isobutyryloxy-7*β*-hydroxynortropane, **1**) showed a molecular ion at *m*/*z* 213.1361, consistent with the molecular formula $C_{11}H_{19}NO_3$ (calcd 213.1364). In the EIMS, the peaks at *m*/*z* 126 [$M^+ - C_3H_7CO_2$], 142 [$M^+ - C_3H_7CO$], and 170 [$M^+ - C_3H_7$] indicated the presence of a hydroxynortropanol skeleton esterified by butyric ($C_4H_8O_2$) acid. The acyl moiety ([$M^+ - 142$], *m*/*z* 71 $[C_4H_7O]^+$) corresponded to a butyryl group, which was further confirmed by the fragments observed



compound	R_1	R_2	R_3	R_4
1	$OCOC^{2'}H(C^{3'}H_3)_2$	H	OH	H
2	OH	H	$OCOC^{2'}H_2Ph$	H
3	$OCOC^{2'}H{=}C^{3'}HPh$ (*cis*)	H	H	CH_3
4	OH	$OCOC^{2'}H(C^{4'}H_3)C^{3'}H(OH)Ph$	OH	CH_3

Figure 1.

at *m*/*z* 43 $[C_3H_7]^+$ and 87 $[C_4H_7O_2]^+$.[13] The presence of a free hydroxyl group at C-7 was inferred by the prominent peak at *m*/*z* 169 [$M^+ - C(6)H_2 - C(7)HOH$]. The ^{1}H NMR spectrum of **1** further confirmed the esterifying moiety to be an isobutyric acid by exhibiting an upfield 6H doublet at *δ* 1.14 ($J_{3',2'} = 5.2$ Hz) assigned to the methyl protons of the isopropyl moiety. A characteristic triplet at *δ* 5.13 ($J_{3eq,2ax} = J_{3eq,4ax} = 4.5$ Hz) indicated the ester substitution at C-3 of the nortropanol skeleton. The presence of a free hydroxyl group at C-7 was confirmed from the doublet of doublets centered at *δ* 4.69 ($J_{7endo,6endo} = 7.4$ Hz, $J_{7endo,6exo} = 2.8$ Hz) due to H-7α. The bridgehead protons appeared at *δ* 3.61 (H-1) and 3.82 (H-5) as a broad singlet and a broad multiplet, respectively. The absence of a characteristic 3H singlet for the *N*-methyl protons in the spectrum indicated a nortropane nucleus. The NH appeared as a multiplet at *δ* 3.02. The ^{13}C NMR spectrum of **1** showed resonances for all 11 carbon atoms in the molecule. The quaternary carbon signal was observed at *δ* 173.4 due to the ester carbon. The signals at *δ* 75.1 and 68.2 were assigned to C-7 and C-3 of the nortropane nucleus. The two equivalent methyl carbons of the isopropyl moiety were observed at *δ* 19.3, while the methine carbon (C-2′) of the acyl group appeared at *δ* 35.2. Complete ^{13}C NMR chemical shift assignments to all atoms

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Table 1. ^{13}C NMR Assignments of the Monomeric Tropane Alkaloids **1**−**4**

carbon	**1**[a]	**2**[a]	**3**[b]	**4**[a]
1	66.5	62.5	59.9	62.1
2	34.1	36.7[c]	36.1	36.2[d]
3	68.2	64.3	67.9	67.4
4	33.5	37.4[c]	36.1	37.9[d]
5	56.7	57.1	59.9	64.8
6	31.9	33.2	26.8	83.7
7	75.1	80.3	26.8	78.6
N-CH$_3$			41.3	40.6
C=O	173.4	169.9	166.3	169.7
2′	35.2	42.3	119.2	54.3
3′	19.3[e]		139.6	91.1
4′				11.9
ipso-C		135.2	132.7	133.5
ortho-C		129.8	128.7	128.4
meta-C		128.3	128.2	128.1
para-C		129.6	130.3	127.3

[a] Spectra recorded at 125 MHz in $CDCl_3$. [b] A spectrum determined at 100 MHz in $CDCl_3$. [c,d] Assignments may be interchangeable. [e] Indicates two-carbon signal.

are presented in Table 1. On the basis of this spectroscopic data, structure **1** was assigned to the new compound.

The new alkaloid **2** was characterized as 3α-hydroxy-7*β*-phenylacetoxynortropane. The IR spectrum showed a strong ester carbonyl absorption at 1716 cm^{-1}. The HREIMS gave a [M]$^+$ ion at *m*/*z* 261.1359 (calcd 261.1364) corresponding to a molecular formula of $C_{15}H_{19}NO_3$. The base peak at *m*/*z* 99 [M$^+$ − C(6)H$_2$ − C(7)HOCOCH$_2$Ph] resulted from the cleavage of the C-7/C-1 and C-6/C-5 bonds.[7] This ion along with a signal at *m*/*z* 142 [M$^+$ − PhCH$_2$CO] indicated the attachment of an ester function at C-7 and the presence of a free hydroxyl group at C-2, C-3, or C-4 of the nortropane nucleus.[7] The presence of a phenylacetoxy group was further inferred from the ions at *m*/*z* 77 [C_6H_5]$^+$, 91 [C_7H_7]$^+$, 119 [C_8H_7O]$^+$, and 135 [$C_8H_7O_2$]$^+$. The ^{1}H NMR spectrum showed a doublet of doublets at *δ* 6.10 ($J_{7endo,6endo}$ = 8.0 Hz, $J_{7endo,6exo}$ = 3.6 Hz) due to H-7α proton, indicating a 7*β*-ester linkage. A broad triplet at *δ* 4.28 ($J_{3eq,2ax} = J_{3eq,4ax}$ = 5.0 Hz) was assigned to the C-3 equatorial (*β*) proton. The absence of a characteristic 3H singlet for the *N*-methyl protons in the spectrum indicated a nortropane nucleus. The NH proton appeared as a multiplet at *δ* 3.02. The ^{13}C NMR spectrum of **2** showed the ester carbonyl carbon signal at *δ* 169.9. The methine carbons of the nortropane skeleton appeared at *δ* 80.3, 64.3, 62.5, and 57.1 assigned to C-7, C-3, C-1, and C-5, respectively. The signals of the phenyl carbons were observed in the range *δ* 128.3−135.2. The complete ^{13}C NMR assignments of compound **2** are presented at Table 1. These spectroscopic observations led to the structure **2** for this new tropane base.

The new alkaloid **3** showed an IR absorption for the ester carbonyl (ν_{max} 1710 cm^{-1}). The HREIMS afforded a [M]$^+$ ion *m*/*z* 271.1568 (calcd 271.1572) corresponding to the molecular formula $C_{17}H_{21}NO_2$. The mass spectrum showed characteristic peaks at *m*/*z* 124 [$C_8H_{14}N$]$^+$ and 140 [C_8H_{14}-NO]$^+$, indicating that the tropane-3-ol skeleton is esterified with cinnamic acid. The presence of characteristic ions at *m*/*z* 148 [PhCH=CHCO$_2$H]$^+$, 147 [PhCH=CHCO$_2$]$^+$, 140 [M$^+$ − PhCH=CHCO], 131 [PhCH=CHCO]$^+$, 103 [PhCH=CH]$^+$, and 77 [C_6H_5]$^+$ was consistent with a cinnamoyloxytropane skeleton.[6,11] The ^{1}H NMR spectrum showed an ester substitution at C-3, and the orientation of the C-3 proton was deduced to be equatorial (*β*) on the basis of coupling constants (t, *δ* 5.16, $J_{3eq,2ax} = J_{3eq,4ax}$ = 4.5 Hz). The acyl residue exhibited signals for *cis* olefinic protons at *δ* 5.92 (COC*H*=CHPh, $J_{2',3'}$ = 12.4 Hz) and 7.03 (COCH=CHPh, $J_{3',2'}$ = 12.4 Hz).[14] The aromatic protons were observed in the region *δ* 7.30−8.08. The ^{13}C NMR chemical shift assignments were found to be consistent with the structure proposed. Thus, the bridgehead carbons (C-1 and C-5) appeared at *δ* 59.9, while the C-3 signal resonated at *δ* 67.9. The signal at *δ* 36.1 was attributed to the C-2 and C-4 methylene carbons. The C-6 and C-7 resonances appeared at *δ* 26.8. The equatorial orientation of the *N*-methyl group was demonstrated by a signal at *δ* 41.3.[15] The signals for C-2′ and C-3′ of the cinnamoyl moiety were observed at *δ* 119.2 and 139.6, respectively. The above data established the structure of the new alkaloid as 3α-*cis*-cinnamoyloxytropane (**3**).

The new alkaloid **4** was characterized as 3α-hydroxy-6*β*-(3′-hydroxy-2′-methyl-3′-phenylpropionyloxy)-7*β*-hydroxytropane. The HREIMS showed a [M]$^+$ ion at *m*/*z* 335.1708 corresponding to the molecular formula $C_{18}H_{25}NO_5$ (calcd 335.1732). A signal appeared at *m*/*z* 222 [M$^+$ − C(6)-HOCOCHMeCHOHPh−C(7)HOH], indicating a tropane-3,6,7-triol skeleton, esterified at C-6 with an acid (acylum ion at *m*/*z* 163).[7] A significant peak at *m*/*z* 113 (M$^+$ − 222) indicated a hydroxyl substitution at C-3 of the tropane nucleus. The fragments observed at *m*/*z* 77 [C_6H_5]$^+$, 107 [C_7H_7O]$^+$, 105 [C_7H_5O]$^+$, 135 [$C_9H_{11}O$]$^+$, 163 [$C_{10}H_{11}O_2$]$^+$, and 179 [$C_{10}H_{11}O_3$]$^+$ resulted from the sequential cleavages of a 3′-hydroxy-2′-methyl-3′-phenylpropionyloxy moiety. The ^{1}H NMR spectrum of **4** showed an upfield 3H doublet at *δ* 1.13 (d, $J_{4',2'}$ = 6.3 Hz) attributed to the methyl group of the acyl moiety. A doublet at *δ* 3.89 ($J_{3',2'}$ = 8.2 Hz) was assigned to the proton at C-3′, while the C-2′ proton appeared as a multiplet at *δ* 3.97. The bridgehead (H-1, H-5) protons appeared at *δ* 3.57 as a broad multiplet. The C-6 endo (α) proton was observed as a doublet at *δ* 5.23 ($J_{6endo,7endo}$ = 7.4 Hz). The free *β*-oriented hydroxyl group at C-7 was inferred from a doublet of the geminal proton at *δ* 3.72 ($J_{7endo,6endo}$ = 7.4 Hz), while the alpha (α) orientation of the hydroxyl group at C-3 was inferred by a triplet at *δ* 4.22 ($J_{3eq,2ax} = J_{3eq,4ax}$ = 5.6 Hz) due to H-3. The ^{13}C NMR spectrum (see Table 1) showed a signal at *δ* 169.7, which was attributed to the ester carbonyl carbon. The signal at *δ* 54.3 was assigned to the C-2′ carbon, while the methyl carbon (C-4′) was observed at *δ* 11.9. A signal at *δ* 91.1 was assigned to the C-3′ methine carbon of the acyl moiety. The C-6, C-7, and C-3 carbons of the tropane ring were observed at *δ* 83.7, 78.6, and 67.4, respectively. The signal appearing at *δ* 40.6 was attributed to the equatorial *N*-methyl carbon of the tropane nucleus.[15] These results led to structure **4** for this compound.

Experimental Section

General Experimental Procedures. Optical rotations were recorded in MeOH and $CHCl_3$ on a JASCO DIP-360 polarimeter. The UV spectra were obtained in MeOH on a Shimadzu UV-240 instrument, while the IR spectra ($CDCl_3$) were determined on a JASCO IRA-1 spectrophotometer. The ^{1}H NMR spectra were recorded in $CDCl_3$ on Bruker AM-300, 400, and 500 NMR spectrometers. The ^{13}C NMR spectra were recorded at 100 and 125 MHz in $CDCl_3$ using TMS as an internal standard. EIMS were recorded on a Varian MAT 312 double focusing mass spectrometer connected to a DEC PDP 11/34 computer system, operating at 70 eV. The HREIMS were obtained on a JEOL JMS-HX110 mass spectrometer. The purities of the samples were checked by TLC on silica gel precoated plates (Merck). Components were detected on TLC plates by UV light or by spraying with Dragendorff's reagent.

Plant Material. Fresh leaves of the plant, a glabrous shrub, were collected from Ja-ela, 12 km from Colombo, Sri Lanka, in November 1992. Voucher specimens of *E. moonii* were

deposited at the PCSIR Laboratories Herbarium, Peshawar, Pakistan, of which the accession number is PES (catalog number 9737). The plant was identified by Mr. Shahid Farooq, taxonomist, PCSIR Laboratories Peshawar, Pakistan.

Extraction and Isolation. Fresh leaves (4 kg) of *E. moonii* were ground and extracted with distilled methanol (12 L) at room temperature for two weeks. The methanolic extract was concentrated under vacuum at 27 °C. The crude gum (160 g) thus obtained was dissolved in distilled water and defatted with hexane. The defatted aqueous extract was then extracted with chloroform at pH 8.0 and 7.0. The chloroform extracts obtained at pH 7.0 (2.40 g) and 8.0 (2.99 g) were subjected to Si gel column chromatography. Mixtures of hexane−chloroform and chloroform−methanol were used as eluents, respectively. Purification of the alkaloids was carried out on preparative TLC cards using the solvent systems mentioned below: system A 95% $CHCl_3$/5% MeOH and ammonia vapor; system B 96% $CHCl_3$/4% MeOH with ammonia vapor; system C 98% $CHCl_3$/2% MeOH and ammonia vapor. Percentage yields are based on the weight of fresh leaves.

3α-Isobutyryloxy-7β-hydroxynortropane (1): white amorphous powder from neutral chloroform extract; yield 1.0 × 10^{-4}% w/w; R_f 0.43 (system C); $[\alpha]^{25}_D$ −13.4° (*c* 0.04, MeOH); UV (MeOH) λ_{max} (log ϵ) 226 (1.47) nm; IR ($CHCl_3$) ν_{max} 3480 (free, OH), 1705 (ester, C═O) cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.14 (6H, d, $J_{3',2'}$ = 5.2 Hz, CH[C*H*$_3$]$_2$), 1.68−2.81 (7H, 3 × m, H_2-2, H_2-4, H_2-6, C*H*[CH_3]$_2$), 3.02 (1H, m, *N*-H), 3.61, (1H, brs, H-1), 3.82 (1H, brm, H-5), 4.69 (1H, dd, $J_{7endo,6endo}$ = 7.4 Hz, $J_{7endo,6exo}$ = 2.8 Hz, H-7endo), 5.13 (1H, t, $J_{3eq,2ax}$ = $J_{3eq,4ax}$ = 4.5 Hz, H-3); ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 1; EIMS (70 eV) *m*/*z* 213 (M^+, 26), 170 (18), 169 (21), 168 (6), 142 (38), 140 (13), 126 (17), 98 (18), 97 (15), 95 (8), 88 (13), 87 (36), 85 (32), 84 (23), 83 (41), 71 (100), 44 (26), 43 (43); HREIMS *m*/*z* 213.1361, calcd for $C_{11}H_{19}NO_3$, 213.1364.

3α-Hydroxy-7β-phenylacetoxynortropane (2): off-white amorphous powder from basic chloroform extract; yield 1.5 × 10^{-4}% w/w; R_f 0.51 (system A); $[\alpha]^{25}_D$ 7.2° (*c* 0.25, MeOH); UV (MeOH) λ_{max} (log ϵ) 264 (2.37), 208 (3.85) nm; IR ($CHCl_3$) ν_{max} 3373 (free, OH), 1716 (ester C═O), 1605 (C═C) cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.96−2.83 (6H, 4 × m, H_2-2, H_2-4, H_2-6), 3.02 (1H, m, *N*-H), 3.72 (2H, s, PhC*H*$_2$COO), 3.86 (1H, brm, H-1), 3.95 (1H, brs, H-5), 4.28 (1H, t, $J_{3eq,2ax}$ = $J_{3eq,4ax}$ = 5.0 Hz, H-3), 6.10 (1H, dd, $J_{7endo,6endo}$ = 8.0 Hz, $J_{7endo,6exoo}$ = 3.6 Hz, H-7endo), 7.46 (2H, dd, *J* = 8.5, 7.8 Hz, meta-H), 7.61 (1H, tt, *J* = 7.8, 1.3 Hz, para-H), 7.96 (2H, dd, *J* = 8.5, 1.3 Hz, ortho-H); ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 1; EIMS (70 eV) *m*/*z* 261 (M^+, 15), 156 (6), 149 (5), 142 (4), 136 (6), 135 (11), 122 (8), 119 (15), 99 (100), 96 (28), 95 (8), 94 (20), 91 (18), 83 (8), 82 (12), 81 (12), 77 (22), 57 (26), 55 (22); HREIMS *m*/*z* 261.1359, calcd for $C_{15}H_{19}NO_3$, 261.1364.

3α-*cis*-Cinnamoyloxytropane (3): white amorphous powder from neutral chloroform extract; yield 8.0 × 10^{-5}% w/w; R_f 0.62 (system A); UV (MeOH) λ_{max} (log ϵ) 275 (4.36) nm; IR ($CHCl_3$) ν_{max} 1710 (ester, C═O), 1605 (C═C) cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.76−2.33 (8H, 4 × m, H_2-2, H_2-4, H_2-6, H_2-7), 2.69 (3H, s, *N*-CH_3), 3.79 (2H, brm, H-1, H-5), 5.16 (1H, t, $J_{3eq,2ax}$ = $J_{3eq,4ax}$ = 4.5 Hz, H-3), 5.92 (1H, d, $J_{2',3'}$ = 12.4 Hz, *cis*-COC*H*CHPh), 7.03 (1H, d, $J_{3',2'}$ = 12.4 Hz, *cis*-COCHC*H*Ph), 7.30−8.08 (5H, m, aromatic H); ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 1; EIMS (70 eV) *m*/*z* 271 (19), 194 (3), 168 (4),167 (2), 147 (3), 140 (16), 131 (29), 124 (100), 122 (17), 105 (22), 103 (32), 97 (14), 96 (75), 95 (18), 94 (32), 84 (12), 83 (65), 82 (53), 77 (27), 67 (24), 57 (16), 55 (18); HREIMS *m*/*z* 271.1568, calcd for $C_{17}H_{21}NO_2$, 271.1572.

3α-Hydroxy-6β-(3′-hydroxy-2′-methyl-3′-phenylpropionyloxy)-7β-hydroxytropane (4): light yellow amorphous powder from neutral chloroform extract; yield 1.3 × 10^{-4}% w/w; R_f 0.34 (system B); $[\alpha]^{25}_D$ −27.4° (*c* 0.17, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 273 (2.38), 216 (4.10) nm; IR ($CHCl_3$) ν_{max} 3350 (OH), 1710 (ester C═O), 1605 (C═C) cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.13 (3H, d, $J_{4',2'}$ = 6.3 Hz, CHC*H*$_3$), 2.02−2.70 (4H, 3 × m, H_2-2, H_2-4), 2.92 (3H, s, *N*-CH_3), 3.57 (2H, m, H-1, H-5), 3.72 (1H, d, $J_{7endo,6endo}$ = 7.4 Hz, H-7endo), 3.89 (1H, d, $J_{3',2'}$ = 8.2 Hz, H-3′), 3.97 (1H, m, H-2′), 4.22 (1H, t, $J_{3eq,2ax}$ = $J_{3eq,4ax}$ = 5.6 Hz, H-3), 5.23 (1H, d, $J_{6endo,7endo}$ = 7.4 Hz, H-6endo), 7.45 (2H, dd, *J* = 8.3, 7.4 Hz, meta-H), 7.59 (1H, tt, *J* = 7.4, 1.2 Hz, para-H), 8.09 (2H, dd, *J* = 8.3, 1.2 Hz, ortho-H); ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 1; EIMS (70 eV) *m*/*z* 335 (5), 258 (3), 228 (2), 222 (4), 200 (3), 179 (2), 172 (3), 167 (10), 163 (3), 156 (3), 148 (41), 139 (3), 135 (6), 124 (100), 123 (13), 122 (73), 121 (7), 113 (18), 107 (7), 105 (90), 97 (18), 96 (12), 95 (15), 94 (13), 85 (15), 84 (15), 83 (34), 82 (23), 81 (17), 77 (67), 67 (17), 57 (48), 55 (39); HREIMS *m*/*z* observed (ion, *m*/*z* calcd) 335.1708 ($C_{18}H_{25}NO_5$, 335.1732), 212.0853 ($C_{10}H_{14}NO_4$, 212.0923), 140.1112 ($C_8H_{14}NO$, 140.1075), 124.1127 ($C_8H_{14}N$, 124.1262), 110.0970 ($C_7H_{12}N$, 110.0970), 94.0664 (C_6H_8N, 94.0656), 84.0815 ($C_5H_{10}N$, 84.0813).

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