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Lilac alcohol epoxide: A linalool derivative in *Actinidia arguta* flowers

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Abstract

Lilac alcohol epoxide (2-(5-methyl-5-(oxiran-2-yl)-tetrahydrofuran-2-yl)propan-1-ol), a previously unreported monoterpene, was identified in the solvent extract of the flowers of seven *Actinidia arguta* genotypes. The diastereomeric lilac alcohol epoxides co-occurred with the lilac aldehydes and alcohols. Another compound, the lilac diol (2-(5-(1-hydroxyethyl)-5-methyl-tetrahydrofuran-2-yl)propan-1-ol) was synthesised as part of our efforts to identify the lilac alcohol epoxide.

Keywords: *Actinidia arguta*; Actinidiaceae; Flowers; Monoterpenes; Lilac alcohols; Lilac aldehydes; Lilac alcohol epoxide (2-(5-methyl-5-(oxiran-2-yl)-tetrahydrofuran-2-yl)propan-1-ol); Lilac diol (2-(5-(1-hydroxyethyl)-5-methyl-tetrahydrofuran-2-yl)propan-1-ol)

1. Introduction

Actinidia arguta (Sieb. Et Zucc.) Planch. ex Miq. var. *arguta* is a kiwifruit species native to northern China, Korea, Siberia and Japan that bears flavoursome smooth-skinned grape-sized fruit (Matich et al., 2003). In the first reported study of the aroma and flavour compounds in genotypes of this species (Matich et al., 2003), the lilac aldehydes (**1**) and alcohols (**2**) (Fig. 1) were identified in the flowers. These compounds have been previously identified in various plant species (Burkhardt and Mosandl, 2003; Jürgens et al., 2002; Kint et al., 1993; Kreck and Mosandl, 2003; Misra et al., 1991; Tollsten and Bergstrom, 1993; Wakayama and Namba, 1974; Watanabe et al., 1974; Wilkins et al., 1993). On the basis of their mass spectra, eight further compounds in *A. arguta* flowers (Matich et al., 2003) (unknowns 156-9 and 204-7, therein) were tentatively identified as diastereoisomeric monoterpenes. These compounds were found only in extracts of *A. arguta* flowers that also contained the lilac compounds, which were the only other components in these samples with multiple chiral centres. This paper reports the isolation and identification of these unknown compounds from the flowers of *Actinidia arguta*.

2. Results and discussion

In contrast to previous harvests (Matich et al., 2003), the unknown compounds (unknowns) were minor components of the solvent extracts. Passage through three silica columns, yielded the unknowns as major components of a fraction which also contained 8-oxolinalool (**4**), 8-hydroxylinalool (**5**), and tyrosol (**6**) as major contaminants (Fig. 2). GC–MS analysis revealed *m*/*z* 143 ($C_8H_{15}O_2$) and 125 ($C_8H_{13}O$) (Fig. 3) which suggested dehydration of an alcohol. Reaction with acetic anhydride and with 2-chlorobenzoyl chloride (Section 4.4.1) produced derivatives without the major fragment ion at *m*/*z* 143. In the acetic anhydride derivative, ions at *m*/*z* 185 and 125 (mass difference of 60) suggested the loss of acetic acid. For the 2-chlorobenzoyl chloride derivative, ions at *m*/*z* 281 and 283, and 125 (mass difference 156 and 158), corresponded to a loss of 2-chlorobenzoic acid.

The field ionisation (FI) mass spectra of the unknowns contained a base peak of 143.1056 ($C_8H_{15}O_2$, $\Delta = 1.6$ mDa), consistent with a lilac alcohol (**2**) minus its ethylene

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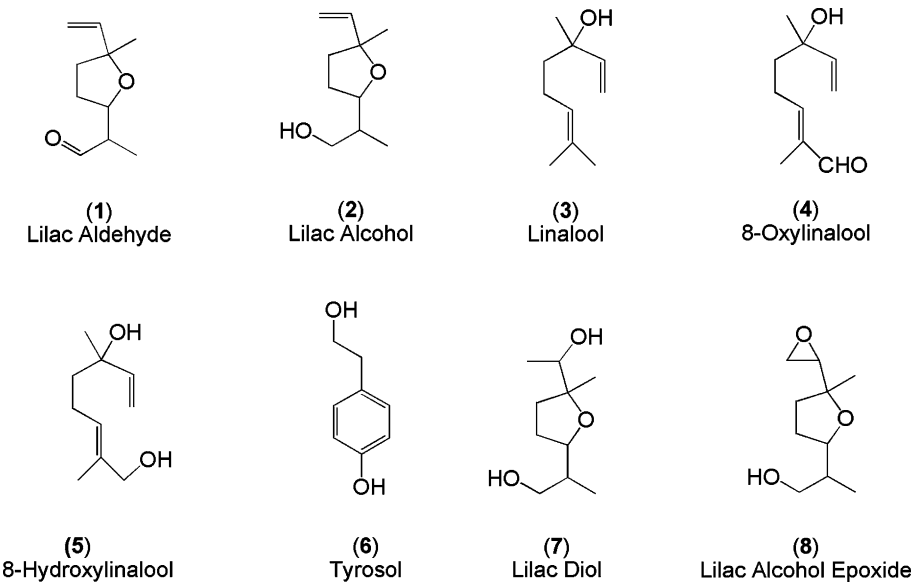

Fig. 1. Structures of compounds identified in extracts of *Actinidia arguta* flowers (excluding (**7**) which was prepared by chemical synthesis).

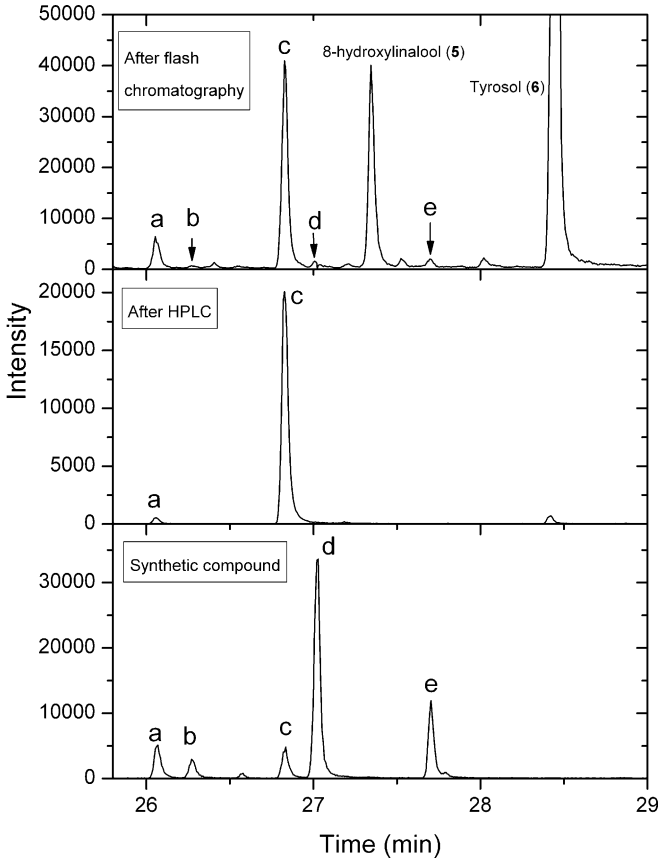

Fig. 2. GC–MS traces of extracts from flowers of *A. arguta* after silica flash chromatography, and after HPLC, and of the synthetic lilac alcohol epoxide (**8**). The isomers of (**8**) resolvable on this column (DB-Wax, Agilent) are labelled a–e.

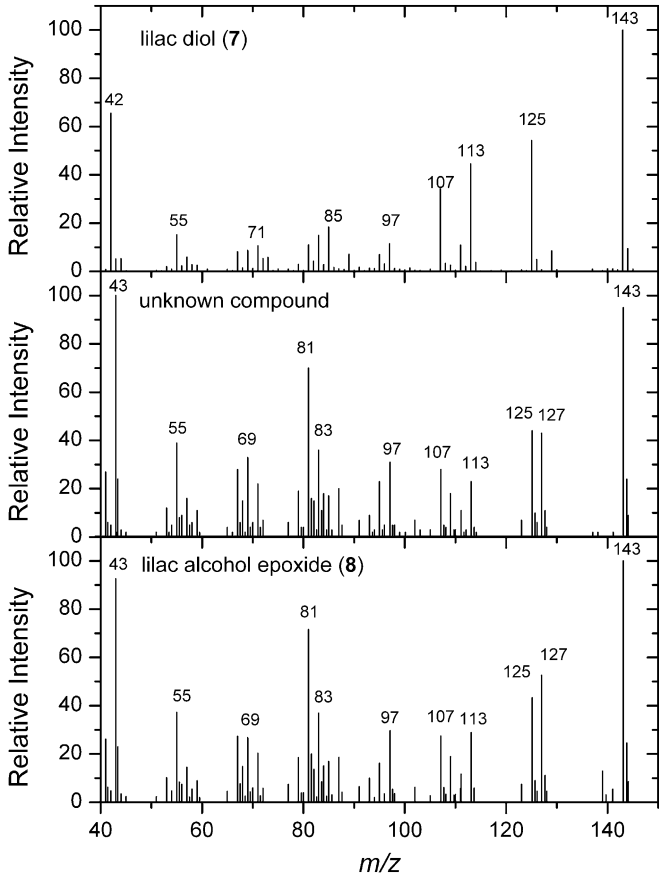

Fig. 3. The electron impact mass spectra of the lilac diol (**7**), the unknowns, and the lilac alcohol epoxide (**8**).

moiety (C_2H_3). The highest mass ion, *m*/*z* 187.1332 ($C_{10}H_{19}O_3$, $\Delta = 0.2$ mDa) could equate to the $(M - H)^+$ ion of a hydrated (M^+ 188) lilac alcohol. The ion of *m*/*z* 143 then corresponds to a loss of 45 Da (C_2H_5O) implying a structure such as (**7**). The longer retention times of the unknown compounds on the polar GC column (Retention indices 2110, 2120, 2150, 2175, 2185, 2210, 2215, and 2280) (Matich et al., 2003) compared with those of the lilac

alcohol diastereoisomers (1750, 1770, 1810, and 1850) was consistent with this thesis.

The lilac diols (**7**) were synthesised from the lilac alcohols (**2**) (Section 4.4.2), but were found to elute from the polar GC column ca. 30 s later than the unknowns. Also, the FI mass spectrum of (**7**) contained *m*/*z* 129, but not the *m*/*z* 127 observed in the unknowns. Diol (7) also produced a very small *m*/*z* 188 (M^+) and a somewhat larger *m*/*z* 189 $(M+1)^+$ species. This suggests that the ion at highest mass produced by the unknowns (*m*/*z* 187) corresponds to $(M+1)^+$.

Further purification of (**8**) by non-aqueous C_{18} column chromatography (Section 4.3.2) removed tyrosol (**6**), but not (**4**) or (**5**). Final separation from these two contaminants (to ca. 87% purity by GC–MS) was by silica preparative HPLC (Section 4.3.3). The ^{1}H NMR spectrum of the unknowns did not contain any vinylic protons, however three coupled one proton double doublets at δ 2.71, 2.8, and 2.95 ppm, indicated a mono-substituted epoxide moiety (Jackman and Sternhell, 1969). A COSY experiment confirmed the coupling of these protons with that at δ 2.71 being coupled to those at δ 2.80 ($J = 5.4$ Hz) and δ 2.95 ($J = 4.0$ Hz) and with δ 2.8 also coupled to δ 2.95 ($J = 2.7$ Hz). The absence of additional proton couplings indicated the adjacent carbon was quaternary. A partial structure that fits these data is a terminal epoxide such as might result from epoxidation of lilac alcohol (**2**). Other NMR data was consistent with this interpretation. A clearly resolved methyl doublet at δ 0.88 ($J = 6.8$ Hz) was coupled to a one proton multiplet (δ 1.98) which in turn was coupled to two tightly coupled protons at δ 3.58 and 3.68 establishing the partial structure $CH_3CH(R)CH_2OH$. A further carbinol proton (δ 4.08) was coupled to two protons at δ 1.78 and 1.92 and through these to a third proton at δ 2.06 establishing a third structural fragment –$CH(OR)CH_2CH$–. These data collectively indicate that the unknowns possess structure (**8**) and probably result from epoxidation of lilac alcohol (**2**).

The ^{1}H NMR spectrum of (**8**), synthesised from the lilac alcohols, by epoxidation using *m*-chloroperbenzoic acid, showed resonances corresponding to those of the natural product but was complicated by the presence of multiple diastereoisomers. EI-GC–MS analysis of this synthetic mixture resolved five of the expected eight peaks, whose mass spectra and retention times proved identical to those found in the isolated sample of (**8**) (Fig. 2) and in the original solvent extract of the flowers. Ratios of isomers in the purified extract did differ from those in the original solvent extract however, because some diastereoisomers were discriminated against as part of the purification process. The FI-GC–MS mass spectrum of both natural and synthetic (**8**) contained a large $(M+1)^+$ ion at *m*/*z* 187 rather than the expected M^+ ion seen by others using this technique (Hancock et al., 2002). The lilac diol (**7**) also showed a strong $(M+1)^+$ ion at *m*/*z* 189, whereas lilac alcohol (**2**) showed only a 10% $(M+1)^+$ contribution with respect to the M^+ ion.

3. Concluding remarks

The unknown monoterpenes detected previously in *A. arguta* (Matich et al., 2003) were shown to be diastereoisomers of lilac alcohol epoxide (**8**). To our knowledge these compounds have not previously been reported in nature. The identification of the absolute stereochemistry of these compounds will require their stereo- and enantioselective synthesis. This is the subject of ongoing research.

4. Experimental

4.1. Plant material

In November 2004, flowers were obtained from seven genotypes of *A. arguta* (Sieb. et Zucc.) Planch. et Miq.var *arguta*. The genotypes sampled were previously designated as A1 ('Hortgem Tahi') (female), A3 (female), A5 (female), A6 (female), A7 (female), A8 (male), and A9 (male) (Matich et al., 2003). All were obtained from the HortResearch orchard in Te Puke, New Zealand. Ca. 2 kg of flowers from all the genotypes (75% were from Hortgem Tahi) was solvent extracted by gentle agitation for 10 min in 2.75 l of 1:1 pentane/Et_2O which had been purified by distillation and passage through a column of activated alumina (Perrin and Armarego, 1988). The extract volume was reduced to 5 ml and dried over $MgSO_4$ in preparation for chromatographic isolation of (**8**).

4.2. Instrumental analysis

GC–MS separations were carried out on an Agilent 6890N GC coupled to a Waters GCT high resolution time of flight (TOF) mass spectrometer using a 20 m × 0.18 mm i.d. × 0.18 μm film thickness DB-Wax (Agilent) capillary column after a 1 min splitless injection. The helium flow rate was 1 ml min^{-1} and the injection port was at 220 °C. The temperature program was 1 min at 35 °C, 5 °C min^{-1} to 150 °C, 10 °C min^{-1} to 240 °C, and hold for 12 min. Accurate mass determinations were carried out using both EI and FI (field ionisation), the latter being a gentle ionisation method more capable of producing molecular ions. In a previous study (Matich et al., 2003), a magnetic sector instrument (VG-70S, Manchester) was used. The TOF machine favours high mass ions, and so *m*/*z* 143 was 98% of the base peak (*m*/*z* 43) intensity (Fig. 3), whereas from the magnetic sector machine it was only 33% (Matich et al., 2003).

4.3. Isolation of compound (*8*) from the solvent extract

4.3.1. Silica flash chromatography

Concentrated solvent extract (5 ml) was eluted through a 100 g × 4 cm dia. silica (Mallinckrodt SilicAR CC-4) column with 200 ml of pentane, and 100 ml each of 5%, 10%, 20%, 40%, 80%, 100% Et_2O in pentane, and finally Et_2O

containing 1% MeOH. Compound (**8**) eluted in the last two solvent fractions by GC–MS. Columns 2 and 3: fractions containing (**8**) were combined, reduced to 1 ml and eluted through a second silica column (25 g × 2 cm dia.) with 50 ml each of 50%, 60%, 70%, 80%, 90%, and 100% Et_2O/pentane with the compounds eluting in the 60–80% Et_2O fractions. The third silica column (25 g × 2 cm dia.) was run using 100 ml of 50% and 200 ml of 60% Et_2O/pentane. Compound **(8)** eluted in the 60% Et_2O fraction.

4.3.2. Reverse phase (C_{18}) flash chromatography

Relevant fractions from the third silica column were reduced in volume to 0.5 ml with 0.5 ml of C_{18} which was added to the top of a 9 × 1 cm dia. C_{18} column previously conditioned with 20 ml each of H_2O, MeOH, DCM, and pentane. Elution was with 30 ml each of 5%, 10%, 20%, and 40% DCM in pentane. Compound (**8**) was found in the early fractions (5–10% DCM), which were combined and reduced in volume to 1 ml.

4.3.3. HPLC isolation of compound (8)

Separation was on a Luna 100 10 μm Silica (2) 250 × 21.2 mm preparative HPLC column (Phenomenex) in a Shimadzu preparative HPLC system at 30 °C. Nine hundred microliters of sample was injected into a mobile phase of 70:30 Et_2O:pentane. The mobile phase composition was ramped up to 100% Et_2O over 20 min and held for 20 min, at a continuous flow rate of 10 ml min^{-1}. Fractions (3 ml) of the eluate were collected for analysis by GC–MS. Compound (**8**) eluted between 30 and 32 min (100% Et_2O). Both the 8-oxolinalool and 8-hydroxylinalool eluted in the latter of the fractions containing the "unknown" compounds. Two of the 3 ml fractions were combined to obtain a sample of (**8**) (ca. 1.9 μl) at a purity of 87% by GC–MS. The yield of compound was thus ca. 0.8 mg kg^{-1} of flowers. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88 (*d*, $J = 6.8$ Hz, CH_3CH), 1.26 (*s*, CH_3C), 1.8–2.14 (weak *m*, $2 \times CH_2CH_2$, $CHCH_3$), 2.71 (*dd*, CH(O)CH_2, $J = 4.0$ and 5.4 Hz), 2.8, (*dd*, CH(O)CH_2, $J = 2.7$ and 5.4 Hz), 2.95 (*m*, –CH(O)CH_2, $J = 4.0$ and 2.7 Hz), 3.58 (*m*, CH_2OH), 3.68 (*m*, CH_2OH), 4.08 (*m*, CHOR); EI-GC–MS 70 eV, *m*/*z* (rel. int.): 143 (95), 127 (43), 125 (44), 113 (23), 107 (28), 97 (31), 83 (36), 81 (70), 69 (33), 67 (28), 55 (39), 43 (100). Accurate mass EI-GC–MS 70 eV, *m*/*z* (*Δ*) fragment: 143.1067 (−0.5 mDa) $C_8H_{15}O_2$, 127.0771 (1.2 mDa) $C_7H_{11}O_2$, 125.0977 (1.1 mDa) $C_8H_{13}O$; FI-GC–MS, *m*/*z* (*Δ*, fragment, rel. int.): 187.1332 (−0.2 mDa, $C_{10}H_{19}O_3$ $(M+1)^+$, 48), 143.1056 (−1.6 mDa, $C_8H_{15}O_2$, 100), 127.0755 (−0.4 mDa, $C_7H_{11}O_2$, 14).

4.4. Chemical syntheses

4.4.1. Derivatisation of extracts of (8)

Two hundred microliters of eluate from the third silica column was gently blown down to 10 μl in a GC vial insert to which 10 μl each of pyridine and of acetic anhydride was added. After 60 min at room temperature the reaction was quenched with water, the organic phase was increased to 150 μl with pentane and dried by passage through $MgSO_4$ in a Pasteur pipette prior to GC–MS analysis, *m*/*z* (rel. int.): 185 (65), 127 (38), 125 (80), 107 (31), 95 (22), 83 (38), 81 (67), 69 (36), 67 (29), 42 (100).

Four hundred microliters of eluate was reduced to 20 μl, to which 10 μl each of pyridine and of 2-chlorobenzoyl chloride was added. The sample volume was increased to 300 μl with Et_2O and washed with 200 μl each of satd. brine and water and dried ($MgSO_4$) prior to GC–MS analysis, *m*/*z* (rel. int.): 281 (31), 168 (19), 141 (37), 139 (100), 127 (25), 125 (48), 111 (30), 107 (13), 83 (15), 81 (38), 69 (10), 67 (13), 43 (30).

4.4.2. Syntheses of candidate compounds

A mixture of the four diastereoisomeric lilac alcohols (**2**) was prepared as described previously (Matich et al., 2003).

Lilac diol (*7*) (*2-(5-(1-hydroxyethyl)-5-methyl-tetrahydrofuran-2yl)propan-1-ol*). The lilac diol (**7**) was prepared as a clear viscous oil (0.10 g, 34% yield) from the lilac alcohols (**2**) by reaction with $Hg(OAc)_2$ in THF/water (Brown and Geoghegan, 1967). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.77 (doublets, $J = 7.2$ Hz, CH_3CH), 0.87 (doublets, $J = 7.2$ Hz, CH_3CH), 1.08 (*m*, $CH_3C(OH)H$; CH_3–C), 1.5 (*m*, CH_2), 1.7 (*m*, CH_2), 2.00 (*m*, CH_2), 2.05 (*m*, CH, CH_2), 3.55 (*m*, CH_2–OH), 3.70 (*q*, CHOH), 4.05 (*m*, CHOR). EI-GC–MS, *m*/*z* (rel. int.): 143 (100), 125 (56), 117 (31), 111 (9), 107 (32), 97 (14), 85 (16), 83 (15), 81 (11), 71 (8), 55 (13), 43 (48). Accurate mass EI-GC–MS, *m*/*z* (*Δ*) fragment: 125.0964 (−0.2 mDa) $C_8H_{13}O$, 143.1070 (−0.2 mDa) $C_8H_{15}O_2$; FI-GC–MS, *m*/*z* (*Δ*) fragment: 189.1510 (1.9 mDa) $C_{10}H_{20}O_3$ $(M+1)^+$, 188.1423 (1.1 mDa) $C_{10}H_{20}O_3$ (M^+), 143.1066 (−0.6 mDa) $C_8H_{15}O_2$, 129.0920 (0.4 mDa) $C_7H_{13}O_2$.

Lilac alcohol epoxide (8) (2-(5-methyl-5-(oxiran-2-yl)-tetrahydrofuran-2-yl) propan-1-ol). To lilac alcohols (**2**) (883 mg, 5.19 mmol) in dichloromethane (DCM, 30 ml) at 0 °C was added *m*-chloroperbenzoic acid (2.0 g, 2.2 equiv.) in portions until the reaction reached 86% completion by GC–MS. Satd. $NaHCO_3$ (50 ml) was added and the organic layer was extracted into DCM (2 × 100 ml). The combined extracts were dried ($MgSO_4$), the solvent removed and the crude product purified by flash chromatography on silica. Elution with EtOAc gave the title compound as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88 (*d*, $J = 6.8$ Hz, CH_3CH), 1.22 (*s*, CH_3C), 1.78 (*m*, CHCHOR), 1.92 (*m*, CHCHOR), 1.98 (*m*, $CHCH_3$), 2.06 (*m*, $CHCH_2$), 2.71 (*dd*, CH(O)CH_2, $J = 4.0$, 5.4 Hz), 2.80, (*dd*, CH(O)CH_2, $J = 2.7$, 5.4 Hz), 2.95 (*dd*, CH(O)CH_2, $J = 4.0$, 2.7 Hz), 3.58 (*m*, CH_2OH), 3.68 (*m*, CH_2OH), 4.08 (*m*, CHOR). EI-GC–MS, *m*/*z*, (rel. int.): 143 (100), 127 (53), 125 (43), 113 (29), 107 (28), 97 (30), 83 (37), 81 (72), 69 (27), 67 (27), 55 (37), 43 (93). Accurate mass EI-GC–MS, *m*/*z* (*Δ*) fragment: 143.1066 (−0.6 mDa) $C_8H_{15}O_2$, 127.0779 (2.0 mDa) $C_7H_{11}O_2$, 125.0976 (1.0 mDa) $C_8H_{13}O$. FI-GC–MS, *m*/*z* (*Δ*) fragment: 187.1334 (0.0 mDa) $C_{10}H_{19}O_3$ $(M+1)^+$,

143.1072 (−0.2 mDa) $C_8H_{15}O_2$, 127.0767 (0.8 mDa) $C_7H_{11}O_2$.

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