Two Novel *ent*-Abietane Diterpenoids from *Isodon xerophilus*

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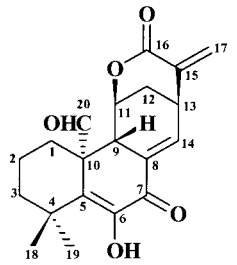
The tow novel *ent*-abietane diterpenoids **1** and **2**, named xerophilusins R and S, respectively (=(11β)-6,11-dihydroxy-7,20-dioxo-*ent*-abieta-5,8(14),15(17)-trien-16-oic acid δ-lactone and (11β,15*S*)-6,11,17-trihydroxy-7,20-dioxo-*ent*-abieta-5,8(14)-dien-16-oic acid δ-lactone, resp.), were isolated from *Isodon xerophilus* (C. J. WU et H. W. LI) H. HARA. Their structures were established by spectroscopic means.

1. Introduction. – *Isodon xerophilus* (C. Y. WU et H. W. LI) H. HARA, a perennial shrub native to Yunnan province [1], belongs to the genus *Isodon*, which has been recognized to be rich in *ent*-kaurane diterpenoids [2][3]. Through bioassay experiments, the AcOEt extract of the leaves of *I. xerophilus* was found to demonstrate significant inhibitory effects on human tumor CEM, MKN-28, Tca8113, T-24, and CA cell lines with $IC_{50} < 29$ μg/ml, which suggested that it has a strong and broad cytotoxicity *in vitro* [4]. Further studies revealed that the AcOEt extract of *I. xerophilus* significantly inhibited the growth of transplanted tumors in mice *in vivo*, while the acute toxicity of it was low [4].

Previous investigations of the AcOEt extracts has already led to the discovery of a series of new and known *ent*-kaurane diterpenoids which are quite relevant to the above antitumor activities and are promising active components of this plant [5–8]. In our ongoing efforts toward searching for minor antitumor *ent*-kaurane diterpenoids, we examined the AcOEt extract of the leaves of *I. xerophilus*, which was collected in Yuanyang County of Yunnan Province in September 1999. As a result, the two novel *ent*-abietane diterpenoids **1** and **2**, named xerophilusins R and S, were isolated and identified. This is the first time that *ent*-abietane diterpenoids were isolated from *I. xerophilus*.

2. Results and Discussion. – Compound **1**, a white powder, showed a molecular-ion peak at *m/z* 342 (M^+) in its EI-MS, consistent with a molecular formula $C_{20}H_{22}O_5$, as confirmed by its HR-EI-MS (M^+ found: 342.1457; calc.: 342.1467) and NMR spectra. Such a molecular formula corresponds to ten degrees of unsaturation. The UV absorptions (309 (3.59), 242 (3.46), 207.5 (3.93) nm) indicated the presence of conjugated systems, including the conjugation of a carbonyl group with an exocyclic methylene group (242 (3.46) nm). The IR spectrum showed the absorptions at 3392 cm^{-1} for OH groups, at 1716 and 1658 cm^{-1} for carbonyl groups, and at 1632 for C=C bonds. The ^{1}H- and ^{13}C-NMR data suggested that compound **1** was a diterpenoid. As all the diterpenoids reported so far from *I. xerophilus* were of the *ent*-kaurane type,



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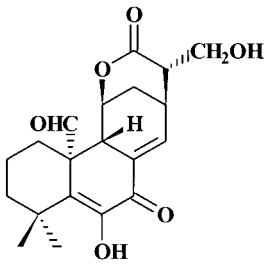

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and based on the characteristic NMR signals of an exocyclic methylene group (δ(C) 127.6 (*t*); δ(H) 6.40 and 5.51 (2*s*, 2 H)) and an aldehyde group (δ(C) 203.3 (*d*); δ(H) 9.59 (*s*, 1 H)), compound **1** was originally proposed to be an *ent*-kaur-16-en-20-al diterpenoid [9]. Normally, *ent*-kaurane diterpenoids show three characteristic ^{13}C-NMR signals of skeletal quaternary C-atoms, *i.e.*, of C(4), C(8), and C(10), in the relatively highfield region. Compound **1**, however, contains only two such quaternary C-atoms, as established by detailed analysis of its ^{13}C-NMR and DEPT spectra. In addition, the C-atoms of the α,β-unsaturated moiety in the five-membered D-ring of *ent*-kaurane diterpenoids generally occur at δ(C) *ca.* 105–118 (*t*) for the exocyclic methylene group and at δ(C) *ca.* 200–212 (*s*) for the C=O group, while compound **1** exhibited the corresponding signals at δ(C) 127.6 (*t*, CH_2=C) and 183.1 and 163.3 (2*s*, 2 C=O). These data suggested that the skeleton of compound **1** corresponds much more likely to that of an abietane-type diterpenoid instead of an *ent*-kaurane-type diterpenoid. Recent studies indicated that, besides *ent*-kauranoids, *ent*-abietane diterpenoids were also present in plants of the genus *Isodon* [10–12]. Interestingly, most of these reported *ent*-abietanoids had an oxygenation pattern similar to those of the corresponding *ent*-kaurane diterpenoids. Presumably, compound **1** biogenetically originates from the *ent*-kaurane diterpenoid ponicidin (**3**) (see below), which was separated in large amounts in this study. Accordingly, compound **1** was established as an *ent*-abietane diterpenoid, finally determined as (11β)-6,11-dihydroxy-7,20-dioxo-*ent*-abieta-5,8(14),15(17)-trien-16-oic acid δ-lactone by extensive 2D NMR (including ^{1}H,^{1}H-COSY, HMQC, HMBC, and ROESY) analysis, and given the trivial name xerophilusin R.

Observed in the ^{1}H-NMR spectrum of **1** (*Table 1*) were two sharp *s* at δ(H) 1.59 and 1.29 for two tertiary Me, a broad *s* at δ(H) 10.75 characteristic of an enol OH, a *s* at δ(H) 9.59 typical for an aldehyde group, a *d* at δ(H) 7.15 (J = 6.6 Hz)) due to an olefinic proton, a pair of *s* at δ(H) 6.40 and 5.51 diagnostic of an exocyclic CH_2=C, and a broad *s* at δ(H) 5.14 arising from an oxygenated CH group. The ^{13}C-NMR and DEPT (*Table 2*) spectra revealed that compound **1** possessed two Me, four CH_2, two CH, one oxygenated CH, two quaternary C-atoms, two C=O, one CH=O, and six olefinic C-atoms (including four quaternary C-atoms, one CH, and one CH_2), which obviously suggested the skeleton of a diterpenoid. In the HMBC spectrum (*Fig. 1*), the ^{1}H,^{13}C long-range correlations of the proton signals at δ(H) 6.40 (*s*) and 5.51 (*s*) of the exocyclic CH_2(17)=C with an ester C=O at δ(C) 163.3 (*s*) assignable to C(16), an olefinic quaternary C-atom at δ(C) 134.8 (*s*) attributable to C(15), and a CH group at δ(C) 33.8 (*d*) due to C(13), indicated an ester carbonyl group conjugated with an exocyclic methylene group, this α,β-unsaturated moiety being attached at C(13). H–C(13) (δ(H) 3.41 (br. *s*)) showed ^{1}H,^{1}H coupling with the olefinic H–C(14) (δ(H) 7.15 (*d*, J = 6.6 Hz)) in the ^{1}H,^{1}H-COSY plot and

Table 1. *^{1}H-NMR Data* (500 MHz) *of Compounds* **1** *and* **2** *in (D$_5$)Pyridine.* δ in ppm, J in Hz.

1		**2**	
H_α–C(1)	2.68 (*m*)	H_α–C(1)	2.68 (*m*)
H_β–C(1)	1.40 (*m*)	H_β–C(1)	1.38 (*m*)
CH_2(2)	1.62 (overlap)	CH_2(2)	1.61 (overlap)
CH_2(3)	1.23 (*m*)	CH_2(3)	1.23 (*m*)
H_β–C(9)	3.05 (*d*, J = 2.2)	H_β–C(9)	3.06 (br. *s*)
H_α–C(11)	5.14 (br. *s*)	H_α–C(11)	5.14 (br. *s*)
H_α–C(12)	1.61 (overlap)	H_α–C(12)	1.48 (*d*, J = 14.0)
H_β–C(12)	2.12 (br. *d*, J = 14.1)	H_β–C(12)	2.63 (br. *d*, J = 14.0)
H_α–C(13)	3.41 (br. *s*, 1 H)	H_α–C(13)	3.18 (br. *s*)
H–C(14)	7.15 (*d*, J = 6.6)	H–C(14)	7.33 (*d*, J = 6.8)
–	–	H_β–C(15)	2.92 (*t*, J = 3.8)
H_a–C(17)	6.40 (*s*)	H_a–C(17)	4.25 (*dd*, J = 10.5, 3.8)
H_b–C(17)	5.51 (*s*)	H_b–C(17)	4.19 (*dd*, J = 10.5, 3.8)
Me(18)	1.59 (*s*)	Me(18)	1.58 (*s*)
Me(19)	1.29 (*s*)	Me(19)	1.29 (*s*)
H–C(20)	9.59 (*s*)	H–C(20)	9.59 (*s*)
HO–C(6)	10.75 (br. *s*)	HO–C(6)	10.71 (br. *s*)

Table 2. *^{13}C-NMR Data* (125 MHz) *in (D$_5$)Pyridine and HMBC Data of Compounds* **1** *and* **2**. δ in ppm.

	δ(C)		HMBC	
	1	**2**	**1**	**2**
C(1)	27.0 (*t*)	27.2 (*t*)	CH_2(2), CH_2(3), H_β–C(9), CHO	CH_2(2), CH_2(3), H_β–C(9), CHO
C(2)	17.0 (*t*)	17.2 (*t*)	CH_2(1), CH_2(3)	CH_2(1), CH_2(3)
C(3)	38.2 (*t*)	38.3 (*t*)	CH_2(1), CH_2(3), Me(18), Me(19)	CH_2(1), CH_2(3), Me(18), Me(19)
C(4)	36.1 (*s*)	36.2 (*s*)	Me(18), Me(19)	Me(18), Me(19)
C(5)	134.4 (*s*)	134.9 (*s*)	CH_2(1), CH_2(3), Me(18), Me(19)	CH_2(1), CH_2(3), H_β–C(9), Me(18), Me(19)
C(6)	149.4 (*s*)	149.5 (*s*)	OH–C(6)	
C(7)	183.1 (*s*)	183.5 (*s*)	H_β–C(9), H–C(14)	H_β–C(9), H–C(14)
C(8)	130.4 (*s*)	130.5 (*s*)	H_β–C(9), H_α–C(11), H_α–C(13), H–C(14)	H_β–C(9), H_α–C(11), H_α–C(13)
C(9)	51.8 (*d*)	52.3 (*d*)	H_β–C(1), H_α–C(11), H_α–C(12), H–C(14)	H_β–C(1), H_α–C(12), H–C(14)
C(10)	55.3 (*s*)	55.4 (*s*)	CH_2(1), H_β–C(9), H_α–C(11), CHO	CH_2(1), CH_2(2), H_β–C(9), H_α–C(11), CHO
C(11)	73.3 (*d*)	73.8 (*d*)	H_β–C(9), H_α–C(12), H_α–C(13)	H_β–C(9), H_α–C(12), H_α–C(13)
C(12)	26.9 (*t*)	23.5 (*t*)	H_β–C(9), H_α–C(11), H_α–C(13), H–C(14)	H_β–C(9), H_α–C(11), H_α–C(13), H–C(14)
C(13)	33.8 (*d*)	28.8 (*d*)	H_α–C(11), CH_2(12), H–C(14), CH_2(17)	H_α–C(11), H–C(14), CH_2(17)
C(14)	135.9 (*d*)	138.9 (*d*)	H_β–C(9), H_α–C(12), H_α–C(13), CH_2(17)	H_β–C(9), H_α–C(12), H_α–C(13)
C(15)	134.8 (*s*)	47.3 (*d*)	H_α–C(13), CH_2(17)	H_β–C(12), H_α–C(13), CH_2(17)
C(16)	163.3 (*s*)	170.9 (*s*)	H_α–C(11), H_α–C(13), CH_2(17)	H_α–C(11), H_α–C(13), H_β–C(15), CH_2(17)
C(17)	127.6 (*t*)	64.2 (*t*)	H_α–C(13)	H_α–C(13), H_β–C(15)
C(18)	27.2 (*q*)	27.4 (*q*)	CH_2(3), Me(19)	CH_2(3), Me(19)
C(19)	26.8 (*q*)	27.0 (*q*)	CH_2(3), Me(18)	CH_2(3), Me(18)
C(20)	203.3 (*d*)	203.6 (*d*)	CH_2(1), H_β–C(9)	CH_2(1), H_β–C(9)

$^1H,^{13}C$ long-range correlations with C(14) (δ(C) 135.9 (*d*)) and the quaternary C(8) (δ(C) 130.4 (*s*)) in the HMBC. The olefinic H–C(14) exhibited a HMBC correlation with C(7)=O (δ(C) 183.1 (*s*)), confirming the presence of the C(14)=C(8) and C(7)=O moieties. The C(5)=C(6) bond was deduced from the $^1H,^{13}C$ long-range correlations of the two tertiary Me (δ(H) 1.59 and 1.29) with the olefinic quaternary C(5) at δ(C) 134.4. The downfield shift of the olefinic quaternary C(6) at δ(C) 149.4 revealed that the only OH group was attached to C(6). Thus, a carbonyl and an enol moiety constituted a conjugated system, which was also verified by the UV absorption at 309 (3.59) nm. The aldehyde group could be easily assigned to C(20) due to the obvious HMBC cross-peaks of CH=O at δ(H) 9.59 (*s*) with C(1) and C(10) (δ(C) 27.0 (*t*) and 55.3 (*s*), resp.)

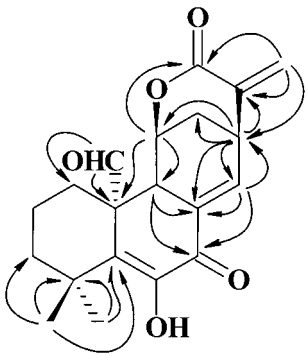


Fig. 1. *Key HMBC correlations of compound* **1**

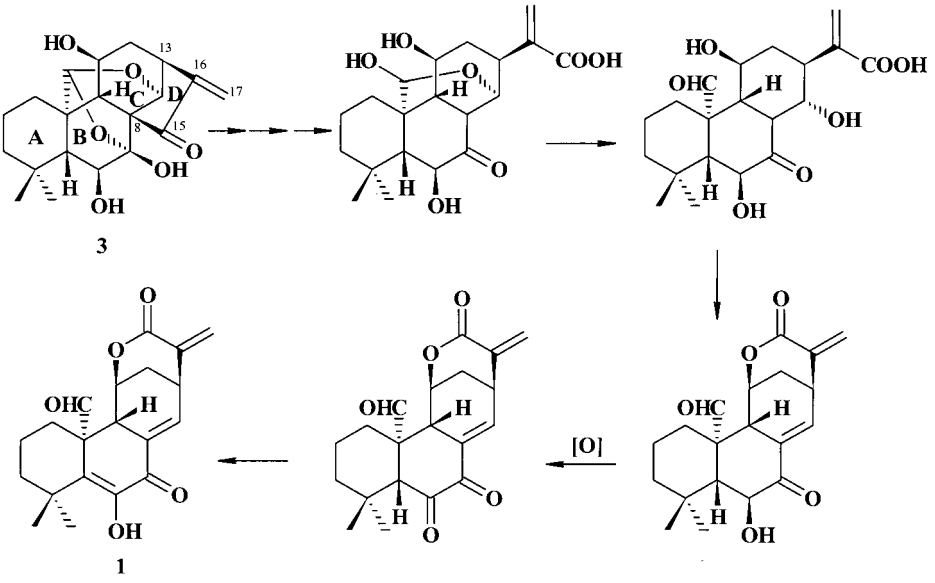
It is noteworthy that the ester C(16)=O at δ(C) 163.3 showed a noticeable HMBC correlation with H–(11) at δ(H) 5.14 (br. *s*), which in turn exhibited $^1H,^1H$ interaction with H–C(9) in the $^1H,^1H$-COSY experiment. This suggested the presence of an O-bridge between C(16) and C(11). With the formation of this δ-lactone ring, the proposed structure exactly satisfies ten degrees of unsaturation. Because of the formation of the δ-lactone in **1**, H–C(11) and H–C(13) could only possess the same orientation. In the ROESY of **1**, the NOE interaction between H–C(11) and H–C(20) established that H–C(11) and H–C(13) were both α-configured.

Compound **1**, as a naturally occurring *ent*-abietane diterpenoid with a novel δ-lactone moiety at C(16) and C(11), is isolated for the first time. Until now, such a type of linkage is unprecedented in natural diterpenoid compounds. A plausible biosynthetic pathway to **1** suggests that the skeleton of compound **1** is biosynthesized from ponicidin (**3**), as shown in the *Scheme*. The possible mechanism for the C–C cleavage reaction of the bond between C(8) and C(15) in **3** would be similar to that proposed previously [10].

Compound **2**, colorless needles, giving a molecular-ion peak at *m/z* 360 (M^+) in the EI-MS, was concluded to have a molecular formula $C_{20}H_{24}O_6$ by the HR-EI-MS (M^+ found: 360.1580; calc.: 360.1573) and the NMR spectra, corresponding to 18 additional mass units (H_2O) as compared to **1**, *i.e.*, one degree of unsaturation less than **1**. Its IR, MS, and 1D- and 2D-NMR data suggested **2** to be another *ent*-abietane diterpenoid, which was structurally very similar to **1**, with the ester C=O group being unconjugated due to the lack of the significant absorption at 242 nm in the UV spectrum. The structure of **2** was established as (11β,15*S*)-6,11,17-trihydroxy-7,20-dioxo-*ent*-abieta-5,8(14)-dien-16-oic acid δ-lactone, named xerophilusin S.

The noticeable differences between the NMR spectra of **1** and **2** were that the signals for the exocyclic CH_2(17)–C(15) group of **1** (see *Tables 1* and *2*) were replaced by the signals for an oxygenated CH_2 (δ(C) 64.2

Scheme. *Postulated Biogenesis of Compound* **1**



(*t*); δ(H) 4.25 and 4.19 (2*dd*, each $J = 10.5, 3.8$ Hz)) and a CH group (δ(C) 47.3 (*d*), δ(H) 2.92 (*t*, $J = 3.8$ Hz)) in **2**, suggesting that **2** was a hydration derivative of the exocyclic C=C bond of **1**. This was supported by the downfield shift of the C(16) signal from δ(C) 163.3 in **1** to 170.9 in **2**. Direct evidence came from the ^{1}H,^{1}H-COSY relationship of CH_2(17)/H–C(15)/H–C(13) and the ^{13}C,^{1}H long-range correlations of CH_2(17) and H–C(15) with C(16) in the HMBC spectrum of **2** (*Table 2*). In the ROESY plot of **2** (*Fig. 2*), the cross-peaks H–C(15)/H–C(14) and CH_2(17)/H_α–C(13) established that H–C(15) was β-oriented and the CH_2OH group at C(15) α-oriented.

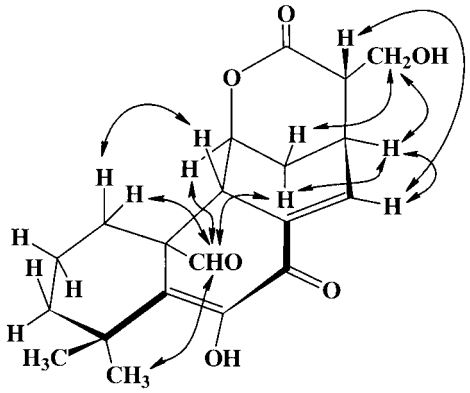


Fig. 2. *Key ROESY correlations of compound* **2**

Both new compounds were assayed for their cytotoxic effects on human-tumor K562 and T24 cell lines according to the methods described in [13], with cisplatin as the positive reference substance. Compound **1** had significant inhibitory activity toward K562 cell lines with an IC_{50} value of 74.450 μg/ml and toward T24 cell lines with an IC_{50}

value of 0.115 μg/ml. Particularly noteworthy, the inhibitory effect of compound **1** on T24 cell lines was even more potent than that of *cis*-platin ($IC_{50} = 1.155$ μg/ml). However, no desirable results were obtained for compound **2**.

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Experimental Part

General. See [10].

Plant Material. The leaves of *I. xerophilus* (C. Y. WU et H. W. LI) H. HARA were collected in Yuanyang prefecture, Yunnan Province, People's Republic of China, in September 1999, and identified by Prof. *Zhong-Wen Lin* at the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, the Chinese Academy of Sciences. A voucher specimen (No. KIB 1999-09-28 Lin) has been deposited in the Herbarium of the Department of Taxonomy, Kunming Institute of Botany, the Chinese Academy of Sciences.

Extraction and Isolation. The dried and powdered leaves (7 kg) were extracted with 95% EtOH (30 l) at 50° for 3 × 24 h, and the extract was filtered. The filtrate was concentrated and partitioned with AcOEt (4 × 10 l). The AcOEt part was evaporated to give 400 g of a residue. A part (390 g) of this extract was subjected to CC (silica gel, 9 × 200 cm, 2400 g; $CHCl_3$, then $CHCl_3/Me_2CO$ 9:1, 8:2, and 7:3, then Me_2CO; TLC monitoring): five crude fractions. *Fr. 2* (27 g) was subjected to CC (1. silica gel (300 g), petroleum ether/AcOEt 2:1; 2. *Lichroprep RP$_{18}$* (100 g), MeOH/H_2O 7:3): **1** (34 mg). *Fr.* 3 (19 g) was further subjected to CC (1. silica gel (200 g), petroleum ether/AcOEt 1:1; 2. silica gel (100 g), hexane/^{i}PrOH 5:1; 3. *Lichroprep RP$_{18}$* (80 g), MeOH/H_2O 65:35): **2** (6 mg).

Xerophilusin R (=*(1S,5R,12aR,12bS)-1,3,4,5,7,9,10,11,12,12b-Decahydro-8-hydroxy-9,9-dimethyl-4-methylene-3,7-dioxo-1,5-methano-12a*H*-napth[1,2-c]oxocin-12a-carbaldehyde*; **1**). Colorless crystals (Me_2CO). M.p. 245–246°. $[\alpha]_D^{22.7} = +374.62$ ($c = 0.33$, MeOH). UV (MeOH): 309 (3.59), 242 (3.46), 207.5 (3.93). IR (KBr): 3392, 2942, 1716, 1658, 1632, 1389, 1305, 1216, 1148, 1135, 1101, 1033, 992, 948, 805, 767. ^{1}H-NMR (C_5D_5N, 500 MHz): *Table 1*. ^{13}C-NMR (C_5D_5N, 125 MHz): *Table 2*. EI-MS (70 eV): 342 (5, M^+), 313 (100), 285 (15), 267 (11), 243 (11), 215 (7), 197 (3), 181 (3), 165 (3), 152 (3), 141 (2), 128 (3), 115 (7), 91 (11), 77 (15), 65 (9), 55 (23). HR-EI-MS: 342.1457 ($C_{20}H_{22}O_5^+$; calc. 342.1467).

Xerophilusin S (=*(1S,4S,5R,12aR,12bS)-1,3,4,5,7,9,10,11,12,12b-Decahydro-8-hydroxy-4-(hydroxymethyl)-9,9-dimethyl-3,7-dioxo-1,5-methano-12a*H*-naphth[1,2-c]oxocin-12a-carbaldehyde*; **2**). Colorless crystal (Me_2CO). M.p. 122–123°. $[\alpha]_D^{15.3} = +91.67$ ($c = 0.30$, MeOH). UV (MeOH): 268 (4.08), 250 (4.16), 203.5 (4.41). IR (KBr): 3412, 2936, 2864, 1713, 1634, 1456, 1373, 1243, 1218, 1056, 981. ^{1}H-NMR (C_5D_5N, 500 MHz): *Table 1*. ^{13}C-NMR (C_5D_5N, 125 MHz): *Table 2*. EI-MS (70 eV): 360 (8, M^+), 331 (100), 313 (17), 301 (10), 285 (14), 267 (10), 243 (19), 215 (18), 199 (11), 189 (15), 173 (15), 161 (10), 141 (10), 128 (12), 115 (13), 105 (17), 91 (26), 77 (19), 69 (17), 55 (39). HR-EI-MS: 360.1580 ($C_{20}H_{24}O_6^+$; calc. 360.1573).

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