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OCCURRENCE AND ORIGIN OF *ENT*-17-HYDROXYKAUR-15-ENE AND *ENT*-17-HYDROXYKAUR-15-EN-19-OIC ACID IN SHOOTS OF *ZEA MAYS*

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Key Word Index—*Zea mays*; Gramineae; *ent*-kauranoids; isolation; synthesis; identification; metabolism; hydroxylation.

Abstract—Previously unidentified metabolites of *ent*-kaur-16-ene and *ent*-kaur-16-en-19-oic acid in shoots of maize are identified, respectively, as *ent*-17-hydroxykaur-15-ene and *ent*-17-hydroxykaur-15-en-19-oic acid by GC-MS comparison with authentic samples. The production of the two latter compounds from the first two substances is analogous to the metabolism of gibberellin A_5 to gibberellin A_3 and provides two further examples, in plants, of hydroxylation at a double bond with rearrangement of the double bond.

INTRODUCTION

Suzuki *et al.* [1] have established the sequence of steps from *ent*-kaur-16-ene (**1**) via *ent*-kaur-16-en-19-oic acid (**2**) to gibberellin A_{12}-aldehyde (**3**) in young shoots of maize (*Zea mays* L.). The ^3H, ^{13}C-labelled substrates for each step were incubated with cubes of the cortex from the lower portion of elongating stem internodes and with homogenates from the same cortical tissue of both light and dark grown plants. The product of each step in the sequence was identified by GC-MS and Kovats Retention Index (KRI). Several unidentified metabolites were also detected and two of them were recognized from GC-MS data as monohydroxylated metabolites of **1** and **2**. This paper presents evidence showing that these two metabolites are *ent*-17-hydroxykaur-15-ene (**4**) and *ent*-17-hydroxykaur-15-en-19-oic acid (**5**).

RESULTS AND DISCUSSION

The mass spectra of the TMSi derivative of the [^{13}C]-metabolite of **1** and of the MeTMS derivative of the [^{13}C]metabolite of **2** contained prominent ions at *m/z* 157, a feature of 15-OTMSi derivatives of *ent*-[^{13}C] kaurenoids [2]. In the course of a GC-MS comparison of the MeTMSi derivatives of the [^{13}C]metabolites of **2** with those of 15α- and 15β-hydroxykaurenoic acids, the presence of 1–2% of the unidentified metabolite from maize was detected in a sample of *ent*-15β-hydroxykaurenoic acid (**8**), previously isolated from the crown gall resin of *Espeletia schulzii* [3, 4]. GC-MS analysis of the gum from the mother liquors of the recrystallization of **8** revealed the presence of 10% of the metabolite of *ent*-[^3H, ^{13}C]kaur-15-en-19-oic acid. Trituration of the gum with dichloromethane gave a solid containing 35% of the metabolite. Flash chromatography of this solid, after methylation, yielded the pure methyl ester of the unknown component. The ^1H NMR of the methyl ester contained a two-proton singlet at δ4.19 ($-CH_2OH$) and a one-proton olefinic singlet at δ5.36, indicating that the structure of the metabolite from **2** was **5**. The methyl ester (**6**) has been described by Wada *et al.* [5] as a product of the photo-oxygenation of **2** but their mp (114–116°) and NMR data for the Me signals (δ0.96 and 1.97) were different from the present mp (131–133°) and δ (0.86 and 1.17). The acid (**5**) has been isolated from other *Espeletia* species by Bohlmann *et al.* [6] and its structure was deduced from the ^1H NMR of the methyl ester (**6**). The methyl ester was described by Bohlmann *et al.* [6] as an oil but contained the same major ^1H NMR signals as the methyl ester isolated in the present work. To confirm the structure of the metabolite of **2**, an independent synthesis of methyl *ent*-17-hydroxykaur-15-en-19-oate (**6**) was undertaken, based on a method by Willis (unpublished) for the conversion of *ent*-gibberellane-16,17-epoxides (**10**) to the corresponding 15-en-17-ols (**11**).

The starting point for the synthesis of **6** was a 13:7 mixture of **2** and *ent*-kaur-15-en-19-oic acid (**12**), obtained from culture filtrates of the fungus, *Gibberella fujikuroi*. This mixture was methylated, then epoxidized with 3-chloroperbenzoic acid in chloroform. The crude product was refluxed with 2,2′-azo-*bis*(2-methylpropionitrile) in dichloromethane and the resultant gum was fractionated by flash chromatography to give the synthetic sample of **6**. The synthetic (**6**) was identical with the methyl ester of the compound isolated from the resin of *E. schulzii* by mixed mp, ^1H NMR and GC-MS. In addition the TMSi derivatives of the synthetic **6** and of the methyl ester of the metabolite of [17-^3H, ^{13}C]-*ent*-kaur-16-en-19-oic acid had the same KRI and the full scan MS (Fig. 1) were

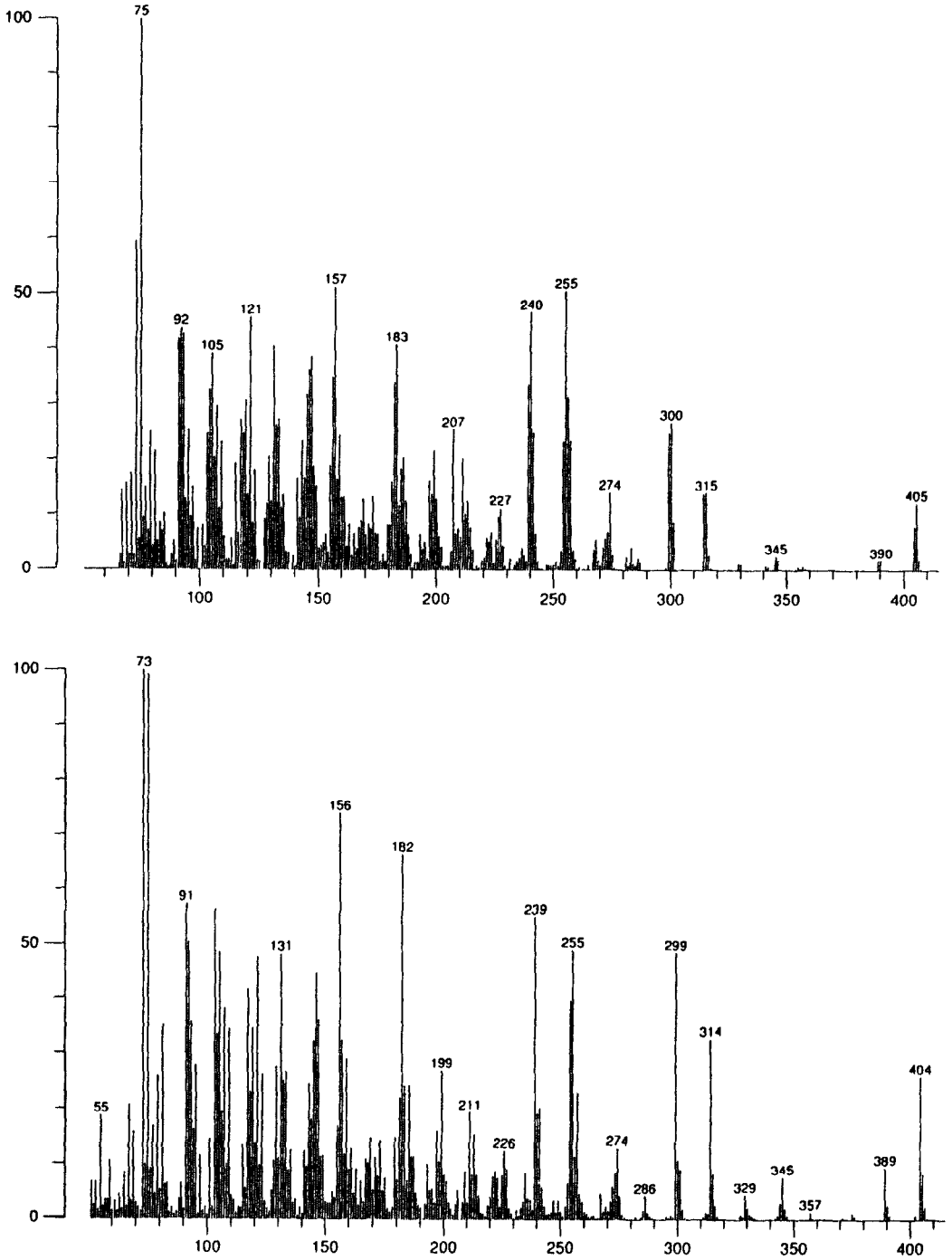
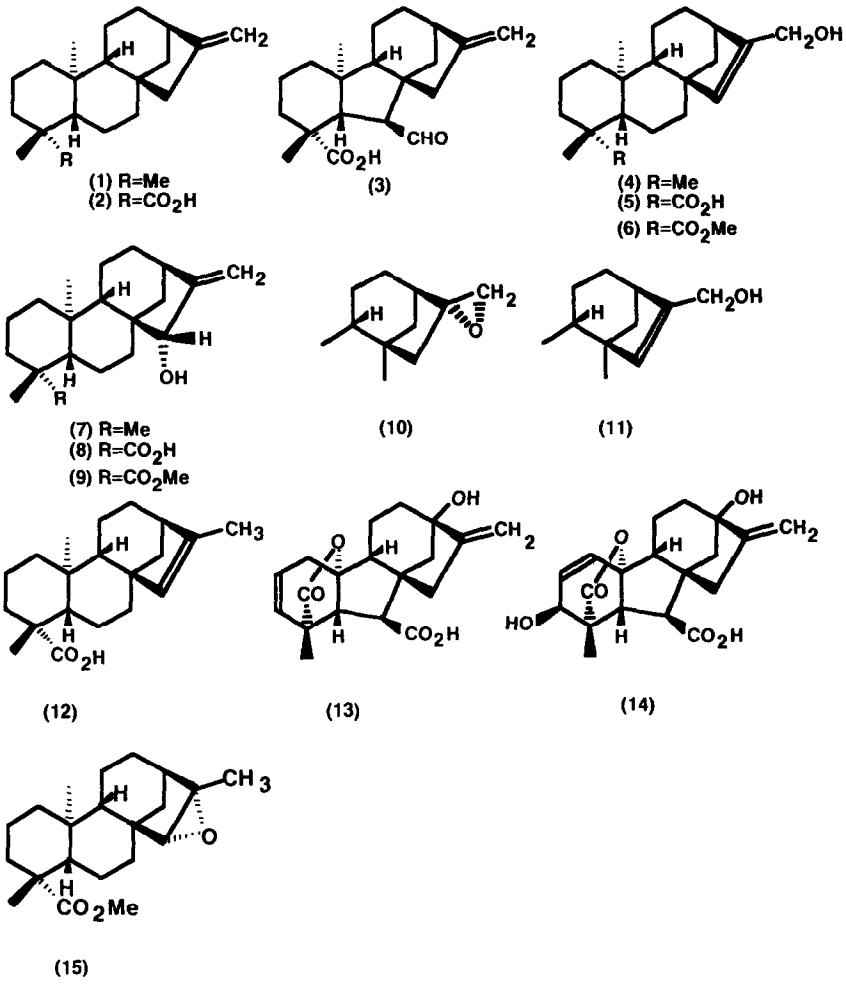


Fig. 1. Full scan GC-MS of the MeTMS derivatives of (upper graph) the metabolite of *ent*-[17-^{13}C, ^{3}H]kaur-16-en-19-oic acid in maize shoots and (lower graph) synthetic *ent*-17-hydroxykaur-15-en-19-oic acid.

identical except that ions containing carbon-17 were displaced by 1 amu in the spectrum of the ^{13}C-labelled metabolite. The mass spectrum (Fig. 1, upper graph) of the maize metabolite showed dilution of the ^{13}C content, originally present in the substrate, [17-^{3}H, ^{13}C]-*ent*-kaur-16-en-19-oic acid, indicating the natural occurrence of **5** in maize stems.

Following the identification of the maize metabolite from [17-^{3}H, ^{13}C]-*ent*-kaur-16-en-19-oic acid as [^{13}C]-*ent*-17-hydroxykaur-15-en-19-oic acid (**5**), the metabolite from **1** was identified as **4** by GC-MS comparison with an authentic sample, previously prepared by singlet oxidation of **1** [7]. The TMSi ethers of the metabolite and the synthetic compound had the same KRI, and full scan



mass spectrum (Fig. 2) were identical apart from the displacement of ions by 1 amu in the ^{13}C-labelled metabolite. In this case also, the ^{13}C content in the metabolite was diluted, providing evidence that **4** occurs naturally in maize stems.

The present results and those of Suzuki *et al.* [1] show that **1** is metabolized to **4** and **7**, that **2** is metabolized to **5** and **8** in maize internodes. They also show the natural occurrence in the internodes of maize shoots of the six compounds **1**, **4**, **7**, **2**, **5** and **8**. The occurrence of **2**, **5** and **8** in the crown gall resin of *E. schutzii* is also established by the present and previous [4] results.

The metabolism of **1** and **2** to their respective 15α-hydroxy derivatives **7** and **8** may be a direct hydroxylation step. However, the metabolism of **1** and **2** to their respective 17-hydroxy derivatives **4** and **5** must involve rearrangement of the 16, 17-double bond to the 15, 16-position either before the hydroxylation step or as part of it; the overall transformation is analogous to singlet oxygenation of olefins [8] which has been realised for **1** [7] and probably for **2** by Wada *et al.* [5], although these authors report incorrect data for their product. The metabolism of **1**–**4** and **2**–**5** is analogous to the conversion of gibberellin A_5 (**13**) to gibberellin A_3 (**14**) by an enzyme preparation from seeds of *Marah macrocarpus* [9].

EXPERIMENTAL

General experimental details are described by Castellaro *et al.* [10].

Isolation of ent-17-*hydroxykaur*-15-*en*-19-*oic acid as the methyl ester from the crown gall resin of* Espeletia schultzii. Extraction, hydrolysis and chromatography of the resin gall of *E. schultzii* was conducted as described in ref. [4], except that 26 g of the crude acid mixt. (not the total 100.5 g as mistakenly stated in ref. [4]) was chromatographed on a column (115 × 5 cm) of silica gel (1.25 kg), packed in petrol. The column was eluted with petrol containing increasing amounts of EtOAc (11 of each solvent mixt., collected in 250 ml frs). Elution with 20% EtOAc gave a mixt. (11.5 g) of *ent*-kaur-16-en-19-oic acid and grandiflorenic acid. Elution with 40% EtOAc gave **8** (3.9 g), crystallized from EtOAc, mp 215–217° (ref. [11] 216–218°). The gum, recovered from the mother liquors of crystallization, was triturated with CH_2Cl_2 and

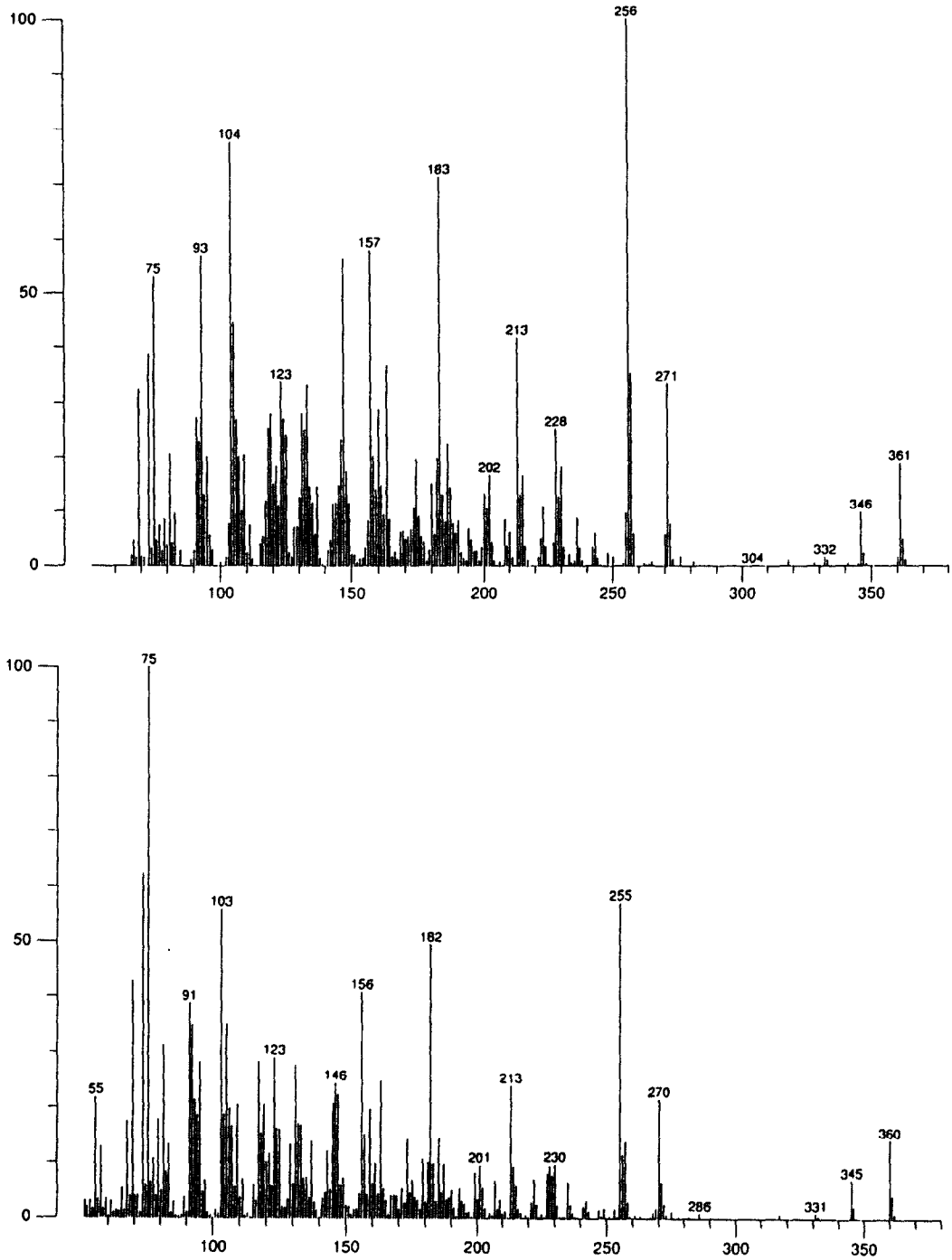


Fig. 2. Full scan GCMS of the TMS derivatives of (upper graph) the metabolite of *ent*-[17-^{13}C, ^{3}H]kaur-16-ene in maize shoots and (lower graph) synthetic *ent*-17-hydroxykaur-15-ene.

filtered to give a solid (262 mg), shown by GC and GC-MS to be a mixt. of **7** (65%) and the metabolite (35%) of **2** from maize. Flash CC of the mixt. (100 mg) on Kieselgel 60 (40–63 μm) and elution with 0–60% EtOAc in *n*-hexane containing 0.5% HOAc did not effect a separation. The methylated (CH_2N_2) mixt. (100 mg) was then subjected to flash CC on a column (150 × 20 mm) of Kieselgel 60 (40–63 μm), eluted with 7 × 100 ml frs each of 5, 7.5, 10, 12.5, 15 and 17.5% EtOAc in *n*-hexane. Elution was monitored by TLC on aluminium-backed precoated Kieselgel 60 with EtOAc:*n*-hexane (1:1) and visualized by spraying with 5% H_2SO_4 in EtOH and heating. Frs 23–30, eluted with 12.5–15% EtOAc, gave Me *ent*-15β-hydroxykaur-16-en-19-oate (**9**, 68 mg), crystallized from MeOH, mp 102–103° (ref. [5] 102–103°); identified by ^{1}H NMR [5] and by GC-MS of the TMSi derivative [2].

Frs 34–38, eluted with 15–17.5% EtOAc, gave methyl *ent*-17-hydroxykaur-15-en-19-oate (**6**, 22 mg), needles mp 131–133° (from MeOH), identical by mmp, ^{1}H NMR and GC-MS of the TMSi derivative with the authentic sample, prepared as described below.

Partial synthesis of methyl ent-17-*hydroxykaur*-15-*en*-19-*oate*. Compound **2** (158 mg), containing 35% of *ent*-kaur-15-en-19-oic acid (**12**), was methylated (CH_2N_2) and the crude product in dry (Na_2SO_4) $CHCl_3$ (10 ml) was treated with 3-chloroperbenzoic acid (98 mg) for 18 hr at room temp. The reaction mixt. was washed with 10% aq. $NaHSO_3$ (2 × 15 ml), with 50% satd aq. $NaHCO_3$ (2 × 15 ml) and with H_2O (2 × 15 ml). The crude recovered product and 2,2′-azo-*bis*(2-methylpropionitrile) (15 mg) in dry (Na_2SO_4) CH_2Cl_2 (10 ml) were heated under reflux for 2 hr. A portion (9 mg) of the crude recovered product was retained for TLC and ^{1}H NMR. The remainder dissolved in the min. vol. of CH_2Cl_2 was applied to a column (150 × 20 mm) of Kieselgel 60 (40–63 μm). After washing with *n*-hexane (100 ml) the column was eluted in steps of 2.5% EtOAc in *n*-hexane (100 ml for each step in 9 frs). Frs 9–15 gave Me *ent*-kaur-16-en-19-oate (11 mg), identified by TLC and ^{1}H NMR. Frs 17–33 gave presumed Me *ent*-kaur-15-en-19-oate 15,16-epoxide (49 mg), mp 123–127°. ^{1}H NMR ($CDCl_3$) δ0.82 (*s*, H_3-20), 1.18 (*s*, H_3-18), 1.43 (*s*, H_3-17), 2.11 (*br s*, H-13), 2.18 (*d*, J = 12.5 Hz), 2.66 (*s*, H-15) and 3.64 (*s*, CO_2Me). Frs 43–48 gave an unidentified mixt. (3 mg). Frs 54–58 gave **6** (22 mg), crystallized from MeOH, mp 131–133°; ^{1}H NMR ($CDCl_3$) δ0.86 (*s*, H_3-20), 1.17 (*s*, H_3-18), 2.08 (*d*, J = 10.3 Hz), 2.17 (*br d*, J = 14.5 Hz), 3.64 (*s*, CO_2Me), 4.19 (*br s*, H_2-17) and 5.36 (*s*, H-15).

GC-MS data. The MeTMSi derivative of the maize-derived metabolite of **2** had KRI = 2566 and MS *m/z* (rel. int.) shown in Fig. 1, upper graph. The TMSi derivatives of **6**, prepared from **2** and isolated from the *E. schutzii* resin, had KRI = 2566 and MS *m/z* (rel. int.) shown in Fig. 1, lower graph. The TMSi derivative of the maize-derived metabolite of **1** had KRI = 2330 and MS *m/z* (rel. int.) shown in Fig. 2, upper graph. The TMSi of **4**, prepared as described in ref. [6] had KRI = 2330 and MS *m/z* (rel. int.) shown in Fig. 2, lower graph.

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