# Rapid and Simple Isolation of Zingiberene from Ginger Essential Oil

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*Received March 4, 1998*

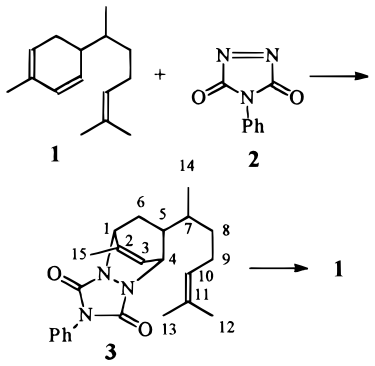
A sesquiterpene-enriched fraction of ginger oil was treated with the dienophile 4-phenyl-1,2,4-triazoline-3,5-dione (**2**), which selectively formed a Diels−Alder adduct with the sesquiterpene hydrocarbon zingiberene (**1**). The adduct was purified by flash chromatography, then hydrolyzed to return zingiberene in good yield and >99% purity.

The sesquiterpene hydrocarbon zingiberene (**1**) [5-(1,5-dimethyl-4-hexenyl)-2-methyl-1,3-cyclohexadiene] has been shown to have a considerable spectrum of biological activity. For example, recent studies have determined its antiviral,[1] antiulcer,[2] and antifertility[3] effects. Furthermore, it has been widely used in cosmetics and fragrances.[4] Zingiberene is a major component of commercially available oil derived from rhizomes of the ginger plant *Zingiber officinale* Roscoe (Musales: Zingiberaceae), but obtaining pure **1** from the mixture of sesquiterpenes in the oil can be problematic. For example, recent publications have described the isolation of zingiberene of low purity[5] or in low yield[1] only after several tedious sequential purification steps.

In connection with the identification of sex pheromone components of the stinkbug *Thyanta pallidovirens* Stål (Hemiptera: Pentatomidae), the males of which produce several sesquiterpene hydrocarbons,[6] we required 100-mg amounts of zingiberene of high purity for laboratory and field bioassays. A search of the literature revealed that zingiberene is generally isolated from essential oils in preparative scale by fractional distillation to produce a fraction enriched in sesquiterpenes, followed by one or more liquid chromatographic steps. However, zingiberene has been selectively removed from ginger oil by the formation of Diels−Alder adducts with maleic anhydride or other powerful dienophiles during the isolation of other sesquiterpene components of ginger oil, such as α-curcumene and sesquiphellandrene.[7] It occurred to us that this strategy of selective removal could be used to obtain zingiberene of high purity by choosing a dienophile that would produce an adduct amenable to controlled degradation, to return the parent diene. 4-Phenyl-1,2,4-triazoline-3,5-dione (PTAD) (**2**), which was developed as a protecting group for endocyclic 1,3-dienes in the B-ring of steroids,[8,9] appeared to be a likely dienophile candidate. In particular, PTAD reacts with 1,3-dienes essentially instantaneously at room temperature to form the Diels−Alder adducts. Furthermore, the resulting adducts are reported to be readily degraded to the parent dienes by either base hydrolysis or reduction with $LiAlH_4$.[8,9]

In our hands, a solution of PTAD in THF was added dropwise to a ginger oil sesquiterpene fraction in THF until the pink color of unreacted PTAD persisted. The amount of PTAD added was not critical, because unreacted zingiberene or excess PTAD were readily removed by chromatography. Thus, after concentration, the mixture was purified by flash chromatography on Si gel to remove the other unreacted hydrocarbons, yielding the pure adduct **3** as a colorless gum. Base hydrolysis of **3**, followed by extraction of the recovered zingiberene into hexanes and Kugelrohr distillation, produced **1** in >99% purity by GC (Scheme 1).

**Scheme 1**




Thus, the method is amenable to the rapid and straightforward preparative scale isolation of zingiberene from ginger oil, without the use of specialized equipment such as spinning band distillation columns or silica LC columns impregnated with $AgNO_3$. Furthermore, the method should be equally applicable to the isolation of related compounds containing endocyclic 1,3-diene moieties, such as 7-epizingiberene[5] or the monoterpene α-phellandrene.

## Experimental Section

Flash chromatography was carried out with 0.04−0.063-mm Si gel (Aldrich Chemical Co., Milwaukee, WI). $^1H$ and $^{13}C$ NMR spectra were recorded in $CDCl_3$ at 300 and 75 MHz, respectively, using a General Electric QE 300 instrument. GC−MS analyses (EI, 70 eV) were performed on a Hewlett−Packard 5890 gas chromatograph interfaced to an Hewlett−Packard 5970B mass selective detector. A DB-5 column was used (30 m × 0.25 mm, J&W Scientific, Folsom, CA; temperature program, 50 °C/0 min, 10°/min−250 °C). HREIMS were obtained with a VG7070 instrument.

Ginger oil (Spectrum Chemical Co., Gardena, CA) was Kugelrohr distilled at 0.1 mmHg. The first fraction (oven temperature <60 °C) containing monoterpenoids was discarded. A second fraction (oven temperature

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S0163-3864(98)00069-X CCC: $15.00 
Published on Web 07/11/1998

60−100 °C) consisted almost exclusively of sesquiterpene hydrocarbons, including α-curcumene (9.2%), zingiberene (46%), sesquiphellandrene (17.4%), and bisabolene (10.1%). A portion of the sesquiterpene fraction (2.0 g, ca. 9.8 mmol of mixed sesquiterpenes) in 20 mL of dry THF was stirred at room temperature, while adding PTAD (0.88 g, 5 mmol; Aldrich) in THF (10 mL) dropwise. The mixture was concentrated and purified by flash chromatography, eluting with 15% $Me_2CO$ in hexanes. The adduct **3** (1.18 g, ca. 69% yield based on percentage of zingiberene in the starting material) was recovered as a colorless gum: $^1$H NMR ($CDCl_3$, 300 MHz) $\delta$ 7.3−7.5 (5H, m, Ph), 5.98 (1H, br d, $J$ = 5.7 Hz, H-3), 5.09 (1H, br t, $J$ = 7 Hz, H-10), 4.97 (1H, dd, $J$ = 5.7, 2.6 Hz, H-4), 4.71 (1H, m, H-6), 2.31 (1H, ddd, $J$ = 13.1, 8.8, 3.5 Hz, H-6), 2.08 (3H, m, H-5, H-9, H-9′), 1.93 (3H, d, $J$ = 1.6 Hz, H-15), 1.70 (3H, s, H-12), 1.63 (3H, s, H-13), 1.5 (1H, m, H-8), 1.25 (2H, m, H-6′, H-8′), 1.10 (1H, m, H-7), 0.88 (3H, d, $J$ = 6.3 Hz, H-14); $^{13}$C NMR (75.48 MHz) $\delta$ 156.43, 156.10, 140.83, 131.84, 131.58, 129.04, 128.05, 125.37, 124.07, 120.59, 55.10, 53.60, 41.34, 36.51, 34.30, 28.57, 25.71, 24.88, 19.40, 17.70, 16.31; EIMS $m/z$ 379 $[M]^+$ (92), 268 (6), 241 (100), 204 (28), 178 (33), 119 (63), 91 (27); HREIMS $m/z$ 379.2258 (calcd for $C_{23}H_{29}N_3O_2$, 379.2260).

The adduct **3** (0.33 g, 0.87 mmol) was refluxed under Ar in 10 mL of 2.1 M KOH in 95% EtOH for 3 h. The cooled mixture was diluted with $H_2O$ and extracted with hexanes (3 × 25 mL). The hexane extracts were washed with saturated aqueous NaCl, dried over $Na_2SO_4$, and passed through a short column of Si gel (ca. 4 cm × 1.5 cm diam), eluting with hexanes, to remove traces of aniline. The solution was concentrated, then Kugelrohr distilled, yielding 143 mg (81%) of zingiberene (**1**), > 99% pure by GC−MS. The mass spectrum and retention times exactly matched those of zingiberene in the crude ginger oil, and the $^1$H NMR spectrum corresponded closely to literature data.[5]

**Acknowledgment.** We thank the California Pistachio Commission for financial support of this work, and the Analytical Services Laboratory, UC Riverside, for obtaining the HRMS.

## References and Notes

(1) Denyer, C. V.; Jackson, P.; Loakes, D. M. *J. Nat. Prod*. **1994**, *57*, 658−662.
(2) Yamahara, J.; Mochizuki, M.; Rong, H. Q.; Matsuda, H.; Fujimura, H. *J. Ethnopharmacol.* **1988**, *23*, 299−304.
(3) Ni, M.; Chen, Z.; Yan, B. *Huadong Huagong Xueyuan Xuebao* **1988**, *14*, 675−679; *Chem Abstr.* **1989**, *111*, 195149w.
(4) Handa, K. L.; Sharma, M. L.; Nigam, N. C. *Perf. Cosmetics* **1963**, *44*, 233−236.
(5) Breeden, D. C.; Coates, R. M. *Tetrahedron* **1994**, *50*, 11123−11132.
(6) Millar, J. G. *Tetrahedron Lett.* **1997**, *38*, 7971−7972.
(7) Connell, D. W.; Sutherland, M. D. *Aust. J. Chem*. **1965**, *19*, 283−288.
(8) Barton, D. H. R.; Shioiri, T.; Widdowson, D. A. *J. Chem. Soc. (C)* **1971**, 1968−1973.
(9) Barton, D. H. R.; Lusinchi, X.; Ramirez, J. S. *Tetrahedron Lett.* **1983**, *24*, 2995−2998.

NP9800699