

## African Elephant Sesquiterpenes

Thomas E. Goodwin,<sup>\*,†</sup> Elizabeth L. Rasmussen,<sup>‡</sup> Ashley C. Guinn,<sup>†</sup> Samantha S. McKelvey,<sup>†</sup> Rohith Gunawardena,<sup>§</sup> Scott W. Riddle,<sup>‡</sup> and Heidi S. Riddle<sup>‡</sup>

Department of Chemistry, Hendrix College, Conway, Arkansas 72032, Department of Biochemistry and Molecular Biology, and Global Change Research Center, Oregon Graduate Institute of Science and Technology (OGI), Portland, Oregon 97291-1000, and Riddle's Elephant and Wildlife Sanctuary, Greenbrier, Arkansas 72058

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GC–MS analysis of extracts from temporal gland secretions of an African elephant has revealed the presence of several farnesol-related sesquiterpenes. Among these are (*E*)-2,3-dihydrofarnesol (**3**), a bumblebee pheromone not seen before in mammals, and a rare component of a Greek tobacco, drimane-8 $\alpha$ ,11-diol (**4**), never observed before in an animal.

Members of the family Elephantidae, African (*Loxodonta africana* L.) and Asian (*Elephas maximus* L.) elephants have a unique subcutaneous gland located on each side of the head about mid-way between the eye and the ear. These are known as temporal glands, owing to their location in the temporal fossa.<sup>1</sup> Aqueous secretions from the temporal gland reach the skin surface through a duct and normally have a pungent and distinctive odor.

Significant quantities of temporal gland secretions (TGS) are usually seen for male Asian elephants during a physiological state termed "musth", a period of high testosterone concentration in the blood, while female Asian elephants secrete quite infrequently. Both sexes of African elephants typically secrete much more often than their Asian counterparts. Although a number of functions have been postulated for TGS, at least one, chemical communication, has been demonstrated rigorously for Asian elephants.<sup>2</sup> In addition, the preovulatory urine of female Asian elephants contains (*Z*)-7-dodecen-1-yl acetate (an essential component of the pheromonal blend of many moth species), which has been conclusively shown to serve as a sexual signal to males.<sup>3,4</sup> Although chemical communication has long been suspected among African elephants, the presence of specific pheromones has not been demonstrated conclusively.

One early study of African elephant TGS focused on analysis of lipids, proteins, and cholesterol,<sup>5</sup> while another reported identification of phenol and *m*- and *p*-cresols by GC–MS.<sup>6</sup> In a seminal study of TGS from 15 African elephants of both sexes, GC–MS analysis of CH<sub>2</sub>Cl<sub>2</sub> extracts revealed not only phenol and *p*-cresol, but also three sesquiterpenes: (*E,E*)-farnesol and two previously unknown farnesol derivatives, (*E*)-3,7,11-trimethyl-2,10-dodecadien-1,7-diol (**1**) and (*E*)-3,7,11-trimethyl-2-dodecen-1,7,11-triol (**2**).<sup>7</sup> The identity of the latter two compounds, which have not been reported elsewhere in nature, was verified by their synthesis from farnesol. These three compounds have been reported subsequently as the only sesquiterpenes among numerous compounds extracted from Asian and African elephant TGS.<sup>8</sup>

The biological activity of these sesquiterpenes has been assessed in several ways. An equimolar mixture of phenol,

*p*-cresol, farnesol, diol **1**, and triol **2** was assayed as a possible chemical repellent against both captive and wild African elephants and was deemed ineffective.<sup>9</sup> In a later report using a different protocol for bioassays, farnesol elicited a greater behavioral reaction from both Asian and African elephants than was shown with controls and produced a surprisingly strong response when compared to musth urine.<sup>8a</sup> Insufficient quantities of farnesol derivatives **1** and **2** precluded a thorough bioassay of these compounds.<sup>10</sup>

The impetus to pursue further analysis of African elephant TGS arose from the realization that much is yet unknown in the chemistry that may connect behavior, physiology, and chemical signaling within this endangered species. As described herein, the search has led thus far to the identification for the first time in a mammal of a major component of the territorial marking perfume of some male bumblebees, as well as the discovery of a tobacco-derived sesquiterpene for the first time in an animal.

In the present research, nonterpenoid constituents of the TGS samples, which were usually found in the greatest amounts, included phenol, *p*-cresol, benzoic acid, *p*-ethylphenol, and 3-phenylpropanoic acid. Triol **2** was observed occasionally as a minor component, while diol **1** and farnesol itself were not found. (*E*)-2,3-Dihydrofarnesol (**3**), known to be a major component of the territorial marker secretion from the labial gland of some species of male bumblebees (*Bombus terrestris* L. and *Bombus huntii* Greene),<sup>11–13</sup> was observed consistently as a TGS component (the absolute configuration of this compound is not known).

Although several of the TGS extracts contained major components (apparently terpenes) that have proven difficult to identify, a search of the Wiley mass spectral library produced a good match with drimane-8 $\alpha$ ,11-diol (**4**)<sup>14</sup> for one of the unknowns. Drimane sesquiterpenes are found frequently in nature in sources as diverse as higher plants and nudibranchs, and often possess intriguing biological activities.<sup>15</sup> Drimane **4**, although synthesized several times,<sup>16</sup> has been reported only once as a natural product, namely as a constituent of a Greek tobacco (*Nicotiana tabacum* L.)<sup>17,18</sup> Worth noting is the fact that compound **4** is isomeric with alcohol **1** and is reasonably imagined as resulting from cyclization of farnesol (or a derivative thereof), followed by hydration.

The identification of sesquiterpenes **3** and **4** in TGS was verified by comparison of the mass spectra of the elephant-

\* To whom correspondence should be addressed. Tel.: (501) 450-1252. Fax: (501) 450-3829. E-mail: goodwin@hendrix.edu.

<sup>†</sup> Hendrix College.

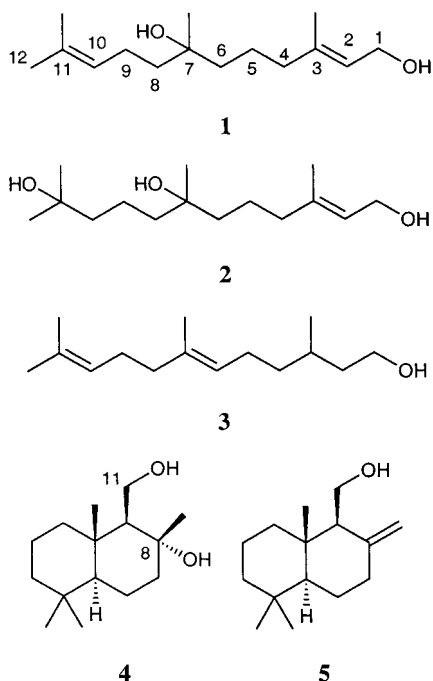
<sup>‡</sup> Department of Biochemistry and Molecular Biology, OGI.

<sup>§</sup> Global Change Research Center, OGI.

<sup>‡</sup> Riddle's Elephant and Wildlife Sanctuary.

derived compounds with those derived from synthetic samples.<sup>19,20</sup> Further confirmation was obtained by spiking TGS extracts with the synthetic compounds before GC-MS analysis to compare retention times and demonstrate peak enrichment.

Although the match was excellent between the mass spectra of the elephant-derived compounds **3** and **4** and synthetic samples, as well as with the 10 most intense peaks listed for tobacco-derived drimane-8 $\alpha$ ,11-diol in the literature (and apparently tabulated as Wiley library spectrum #51998),<sup>17</sup> a perplexing inconsistency arose for diol **4**, which led initially to confusion. In a review article about the mass spectra of biodegraded isoprenoids that occur naturally in tobacco,<sup>21</sup> the spectrum labeled "8,11-drimanediol" does not exactly match those discussed above. Although the spectra are quite similar, one notable discrepancy is the inversion of intensities for two diagnostic peaks. In the anomalous mass spectrum, the intensity ratio of the  $m/z$  137 fragment over the  $m/z$  164 fragment is 3.3/1, while the same ratio is 0.3/1 for the elephant-derived compound. In fact, the  $m/z$  137 ion is not among the 10 most abundant fragments for any reported mass spectrum of compound **4**, except for that given by Enzell and Wahlberg.<sup>21</sup> It is possible that under the conditions used to vaporize the drimane-8 $\alpha$ ,11-diol sample for the mass spectrum used in the review article,<sup>21</sup> a portion of the compound may have dehydrated to provide, among other products, albicanol (**5**). Because the mass spectrum of albicanol exhibits a base peak at  $m/z$  137,<sup>22</sup> the percentage of this fragment in the resulting composite mass spectrum would increase.



The discovery among widely diverse organisms of identical natural products often serving similar functions is intriguing, yet hardly unprecedented.<sup>4</sup> Although the search to identify other interesting components of African elephant TGS is actively ongoing, many questions are as yet unanswered. Among these are the following. Is the source of these sesquiterpenes the elephant metabolic processes per se, microbial alteration<sup>23</sup> within the temporal gland or elsewhere, diet, or another origin? Are sample-to-sample variations in the exact mix of extractable organic com-

pounds in TGS due to collection and preservation techniques, variations in diet, changes in the physiology of the elephant from season to season or year to year, or something else? How will the constituents of TGS vary from elephant to elephant, from male to female, and from captive to wild elephants as the sampling database is expanded? Are any of the organic components of African elephant TGS used in chemical communication, and if so, how? It is expected that answers to some or all of these questions will result from additional research efforts which are underway.

## Experimental Section

**General Experimental Procedures.** During initial studies, TGS samples were placed on ice immediately after collection, then stored in a  $-20^{\circ}\text{C}$  freezer until analysis. Later samples were frozen immediately with liquid nitrogen, transported on dry ice, and stored at  $-70^{\circ}\text{C}$ . After thawing, samples were extracted three times with a three-fold volume excess of  $\text{CH}_2\text{Cl}_2$ . The combined extracts were dried over anhydrous  $\text{Na}_2\text{SO}_4$  and concentrated to approximately  $100\ \mu\text{L}$  with a gentle stream of dry Ar or  $\text{N}_2$ .

**Animal Material.** Routine accessibility to a healthy, well-managed, and well-behaved maturing male African elephant (aged 12 to 15 during time of sampling) made noninvasive collection of TGS rapid and safe. Samples, ranging in size from a few hundred microliters to a few milliliters, were collected in small, conical glass flasks at irregular intervals over a period of three years by external manual palpation around the temporal glands of the unsedated elephant. Dates of sample collection were as follows: July and August 1995; June 1996; and April, May, June, July, and September 1998. Samples were frozen until extraction with  $\text{CH}_2\text{Cl}_2$ , concentration, and analysis by GC-MS.

**Gas Chromatography-Mass Spectrometry.** Dried and concentrated  $\text{CH}_2\text{Cl}_2$  extract of TGS ( $0.5\text{--}1.5\ \mu\text{L}$ ) was introduced onto a GC-MS via an on-column injector system. GC analysis was performed using a Hewlett-Packard 5890 Series II instrument equipped with a  $60\ \text{m} \times 0.32\ \text{mm}$  (i.d.) capillary column (HP-1,  $1\ \mu\text{m}$  film thickness). The column oven was temperature programmed from  $35$  to  $310^{\circ}\text{C}$  at  $5^{\circ}\text{C}/\text{min}$ . Mass spectra were obtained with a Hewlett-Packard 5971A mass selective detector. Initial searches were carried out using an NBS 75 K Hewlett-Packard MS Chem Station library. These were manually rechecked with NIST/EPA/NIH Mass Spectral Data Base Version 4.01.

**Reference Compounds.** (*E*)-2,3-Dihydrofarnesol (**3**) was prepared via  $\text{NaBH}_4$  reduction of the corresponding aldehyde, which was prepared by a literature procedure.<sup>19</sup> Drimane-8 $\alpha$ ,11-diol (**4**) was kindly provided by Professors A. Barrero and M. Cortes.<sup>20</sup>

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University of Berlin). Finally, this research was made possible by the cooperation of Solomon, one of 10 elephants at Riddle's Elephant and Wildlife Sanctuary.

**Supporting Information Available:** Mass spectral comparisons of synthetic samples with elephant-derived (*E*)-2,3-dihydrofarnesol (**3**) and drimane-8 $\alpha$ ,11-diol (**4**). This material is available free of charge via the Internet at <http://pubs.acs.org>.

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