Caste-Specific Tyramides from Myrmicine Ants†

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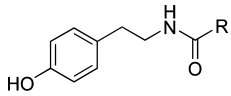
Analysis of the extracts of male ants of *Monomorium minimum* and *Monomorium ebeninum* by GC−MS and GC−FTIR revealed the presence of tyramides **2** and **4c**, for which the structures were established by comparison with synthetic samples. These compounds and their analogues **1** and **3** were also found in males of other *Monomorium* species, males of *Myrmicaria opaciventris*, and males of several *Solenopsis (Diplorhoptrum)* species. Vapor-phase FTIR spectra revealed critically important structural clues to two of the tyramides, which had methyl branching in the tyramide acyl moiety. Tyramide **4c** exhibited a strong intramolecular amide *N*H hydrogen bond where an α-keto group was deduced to be present in the acyl moiety and also showed the overlap of this ketone group frequency with that of the amide $\nu_{C=O}$. The biological function of these compounds is uncertain; however, their role in ant-mating behavior may be suggested by a large body of evidence.

Ring-saturated nitrogen heterocycles are well-known components of the venoms of ants in the subfamily Myrmicinae, particularly in the genera *Megalomyrmex*, *Monomorium*, *Myrmicaria*, and *Solenopsis*.[1–4] While different species of ants may have some of the same alkaloids, the alkaloid composition of a particular species seems to be characteristic, varying only with the age of the ants.[5] In several cases, a marked difference has been observed between the venom alkaloids produced by queens and those produced by workers of a particular species.[6,7] The venom alkaloids found in the workers, and in some cases the queens, of the species reported in this investigation, *Monomorium minimum*, *Monomorium pharaonis*, *Monomorium floricola*, *Myrmicaria opaciventris*, *Solenopsis molesta*, and *Solenopsis maboya*, have been described, while those of *Monomorium ebeninum* are very similar to those of *M. miminum*.[1,7–9] These compounds are conspicuously absent in the extracts of all male ants in these genera, which do not have venom glands. Herein we describe, however, not the presence of alkaloids but the occurrence of a set of acylated tyramines (**1**−**3** and **4c**) from males of nine species of the genera *Monomorium*, *Myrmicaria,*, and *Solenopsis (Diplorhoptrum)*. Compounds **1**−**3** have been previously reported from marine bacteria,[10] while the α-ketoamide **4c** is a new compound.



1 R = Et
2 R = *n*-Pr
3 R = 2-Pr
4c R = $COCH_2CH(CH_3)_2$

Results and Discussion

In the summers of 1995 and 1996, males of the small black ant *M. minimum* Buckley, common in the eastern United States, were collected from nests in Lexington, VA. The initial gas chromatography−mass spectrometry (GC−MS) analysis of CH_2Cl_2 extracts of these specimens revealed the presence of two compounds, **2** and **4c**, for which the mass spectra had *m*/*z* = 120 (100) and a significant ion at *m*/*z* 107. In one experiment, trisection of *M. minimum* revealed by GC−MS that these compounds occur in the gaster of the ants. Subsequently, males of the other species listed in Table 1 were examined. Trisection of *M. ebeninum* showed that these compounds also were found only in the gasters of the ants. Extracts of whole ants only were available for the remaining species. Trisection of myrmicine workers has never shown tyramides.

Table 1. Distribution of Tyramides from Myrmicine Ants

species	dates collected	1	2	3	4c
M. minimum	1995 and 1996		*		*
M. ebeninum	1996 and 2003		*		*
M. floricola	1999		*		
M. pharaonis	1999				*
M. opaciventris	2003		*		
S. nr. *molesta*	1993	*			
Solenopsis #11 PR	1995			*	
Solenopsis #78 PR	1998			*	
S. maboya	1999 and 2000			*	

In *M. minimum*, the first component had the following. GC−FTIR spectrum: ν_{max} 3652, 3466, 3016, 2968, 2936, 2887, 1707(s), 1613, 1500(s), 1443, 1332, 1256, 1173, 1106, 821 cm^{-1}. EIMS [M^+] *m*/*z* 207 (2), 164 (1), 121 (13), 120 (100), 107 (41), 91 (1), 88 (24), 77 (22), 71 (36), 55 (5), 43 (77), 41 (25); HRMS *m*/*z* 207.1254, calcd for $C_{12}H_{17}NO_2$, *m*/*z* 207.1259. Its MS spectrum and GC retention time (20.0 min) were identical with those of an authentic sample of *N*-[2-(4-hydroxyphenyl)ethyl]butanamide (**2**).[11]

The second and roughly equimolar component at *Rt* 21.0 min had the following. EIMS: [M^+] *m*/*z* 249 (2), 234 (1), 206 (1), 164 (4), 121 (35), 120 (100), 107 (22), 91 (9), 85 (13), 77 (18), 57 (40), 43 (8), 41 (20). Initially, it was assumed that this component was a three-carbon homologue of **2**; however, the high-resolution mass spectrum (HRMS *m*/*z* 249.1361, calcd for $C_{14}H_{19}NO_3$, *m*/*z* 249.1365) indicated the presence of a third oxygen and another double-bond equivalent. GC−FTIR of this component had ν_{max} 3652, 3429, 3019, 2968, 2938, 2885, 1707(s), 1614, 1513(s), 1439, 1365,1336, 1258, 1172, 1102, 820 cm^{-1}. The frequency (3429 cm^{-1}) of the secondary amide *N*H of this component was shifted to a lower frequency by 37 cm^{-1}, suggesting that an intramolecular hydrogen bond was present. The lack of an obvious IR absorption

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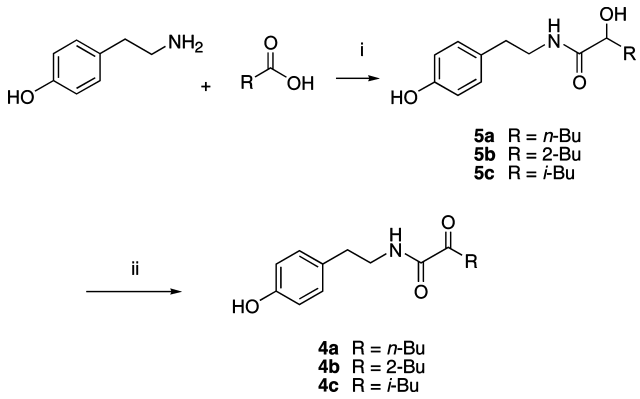
Scheme 1. Synthesis of 2-Ketohexanoyltyramines **4a**−**4c**



[i] EDCI, HOBT, DMF.
[ii] Jones Reagent or O_2, V_2O_5, toluene, Δ.

for the third oxygen, e.g., a ketone or epoxide functionality, presumed to be in the acyl portion of the amide, was perplexing. The six double-bond equivalents determined from HRMS in comparison with the five found for **2** could be consistent with (1) a hydroxy group and double bond, (2) an epoxide, (3) a ketone, or (4) a ring with an ether oxygen. No additional hydroxy frequency was observed in addition to the sharp phenolic ν_{OH} at 3652 cm $^{-1}$ nor was any internal/terminal olefinic $\nu_{=CH}$ absorption noted, ruling out the first possibility. No unambiguous absorptions for an epoxide could be ascertained, but an α-keto group in the acyl moiety could not be excluded because a few examples that we were aware of have this absorption coupled to the ester or amide absorption and sometimes are seen as a single absorption band.[12] Nonetheless, it was assumed that an ether ν_{CO} might be present but was difficult to detect by IR spectroscopy, and consequently three tyramides were synthesized having a cyclic methyl, tetrahydrofuranyl ether, a tetrahydropyranyl ether, or an α−*β*-epoxide group, all of which might be expected to provide a hydrogen-bond receptor for the observed amide *N*H donor. This synthetic work and that of other preliminary structural possibilities for the *m*/*z* 249 tyramide will be reported elsewhere. None fit the GC−MS or GC−FTIR behavior of the *m*/*z* 249 tyramide so the structural possibility (4) above, although biosynthetically attractive, was made less likely. Not all permutations of cyclic ethers were synthesized because about this time we discovered by a close examination of the amide I/amide II band ratio in the *m*/*z* 249 tyramide IR spectrum the likely presence of a ketone group in the acyl group, most probably in the α position. This conclusion arose from the observation that the ratio of the amide I to amide II bands in the natural molecular weight 249 tyramide was appreciably greater than that seen in **2** as well as those seen with *all* of the other non-α-ketotyramides, where the amide II band at 1500−1505 cm^{-1} is significantly more intense than the amide I band. We concluded, therefore, that the stretching vibration of the α-keto group coincides with that of the amide $\nu_{C=O}$ group, enhancing its intensity, and is an example of the "coupling effect" that has been reported for amides.[13] The above-mentioned shift in the amide *N*H to a lower frequency, which suggested hydrogen bonding to an oxygen function, is consistent with an α-keto group being a hydrogen-bond acceptor.

Small amounts of the α-ketoamides (**4a**−**4c**) for a GC−MS and GC−FTIR comparison with the natural α-ketoamide were prepared by a careful oxidation of the corresponding α-hydroxyamides (**5a**−**5c**) with the Jones reagent at 0 °C (Scheme 1). The hydroxyamides **5a**−**5c** formed nearly quantitatively by coupling 2-hydroxyhexanoic acid, 2-hydroxy-3-methylpentanoic acid, or 2-hydroxy-4-methylpentanoic acid[14] with tyramine in the presence of *N*-ethyl-*N*-[3-(dimethylamino)propyl]carbodiimide/1-hydroxybenzotriazole. Numerous oxidation methods of the hydroxyamides were attempted, including PCC, PDC, CrO_3 on Celite, chromic acid/ether, Swern oxidation, and Dess−Martin periodinane, but the Jones reagent at 0 °C for 10 min, although providing low yields (10−20%), gave clean samples of the α-ketoamides after flash chromatography. A great improvement in the oxidations of **5a** and **5c** was achieved by air oxidation with a catalytic amount of V_2O_5 in toluene at 90−100 °C,[15] where **4a** and **4c** were obtained, respectively, in ca. 60% yield after chromatography.

The straight-chain analogue (**4a**) was first synthesized and found to be nonidentical with the natural tyramide, but it was recognized by close inspection of the ν_{CH} region (see Figure 1) that the asymmetric vibration at ca. 2968 cm^{-1} relative to the ν_{CH} methylene stretching vibration at 2938 cm^{-1} indicated that another methyl group was clearly present in the acyl moiety of the natural tyramide. This "methyl-counting" method had earlier been applied by one of us (H.M.G.) to the assignment of a tentative structure to a novel homopumiliotoxin alkaloid **249F** found in an Argentine toad.[16]

The C−H bending vibrations at ca. 1385 cm^{-1} gave no clear evidence of a *gem*-dimethyl group, so a 2-keto-3-methylpentanoyl tyramide (**4b**) was then synthesized. Although very similar in its MS and IR spectra, it was not identical with the natural tyramide, leaving 2-keto-4-methylpentanoyl tyramide (**4c**) as the remaining likely structural possibility for the *m*/*z* 249 tyramide. Synthesis of 2-keto-4-methylpentanoyl tyramide (**4c**) finally revealed that the natural *m*/*z* 249 tyramide possesses this same structure, despite the fact that the *gem*-dimethyl absorption, expected for such an "iso" structure, is not unambiguously evident. The MS spectrum, GC retention time, and GC−FTIR spectrum of the natural tyramide matched that shown for **4c**, as depicted in Figure 1.

Evidence for oxygen functionality at the α position of the tyramide acyl moiety was also deduced from the EIMS of the natural *m*/*z* 249 tyramide, where a cleavage between the acyl amide C=O and the adjacent ketone carbon is seen, giving an *m*/*z* 85 (12%) fragment, and a related weak but diagnostic fragment at *m*/*z* 164 (2.4%) is seen as an indication that both cleavage fragments carry charge. No cleavage for **4c** was observed between the acyl carbonyl and the amide nitrogen, yielding a minor or even significant fragment ion at *m*/*z* 130, which occurs in *all* of the other tyramides lacking an α- keto- or α-hydroxyl substituent. The major fragments of all of the tyramides are governed by cleavage at the benzylic position to afford the *m*/*z* 107 ion (ca. 40%) and a McLafferty rearrangement to yield as the base peak the *m*/*z* 120 ion, having a *p*-hydroxystyrene structure.

After the structures of **2** and **4c** were established in males of *M. minimum* and *M. ebeninum*, extracts of the other male ants listed in Table 1 were examined and the structures of tyramides **1**−**3** were determined by spectroscopic analysis and comparison with synthetic material.[11,17] These tyramides have not been detected in extracts of workers or queens of any of the species in Table 1 over the many years of this study.

While the biological role of these tyramides is as yet undetermined, their occurrence exclusively in males of the species examined suggests that they may have a function in the mating behavior in these ants, where a fair amount of field research already exists that indicates a role for the involvement of some pheromone, such as these compounds.[18] Male-specific compounds have been reported in other ant subfamilies for many years.[19,20] Ant-mating behavior can be divided into a female-calling syndrome and a male-aggregation syndrome. In the latter, males first attract additional males and the buildup of pheromones later attracts females.[21] For example, in a species of the myrmicine genus *Crematogaster*, the males occupy the top portion of the swarm and the females are attracted to that area and occupy the bottom portion of the swarm.[22]

Of the genera examined here, in *Monomorium* (=*Chelaner*) both syndromes have been observed for particular species.[23] In *M. opaciventris*, it has been observed that the winged males leave the nest first, followed by the alate females, and mating takes place in

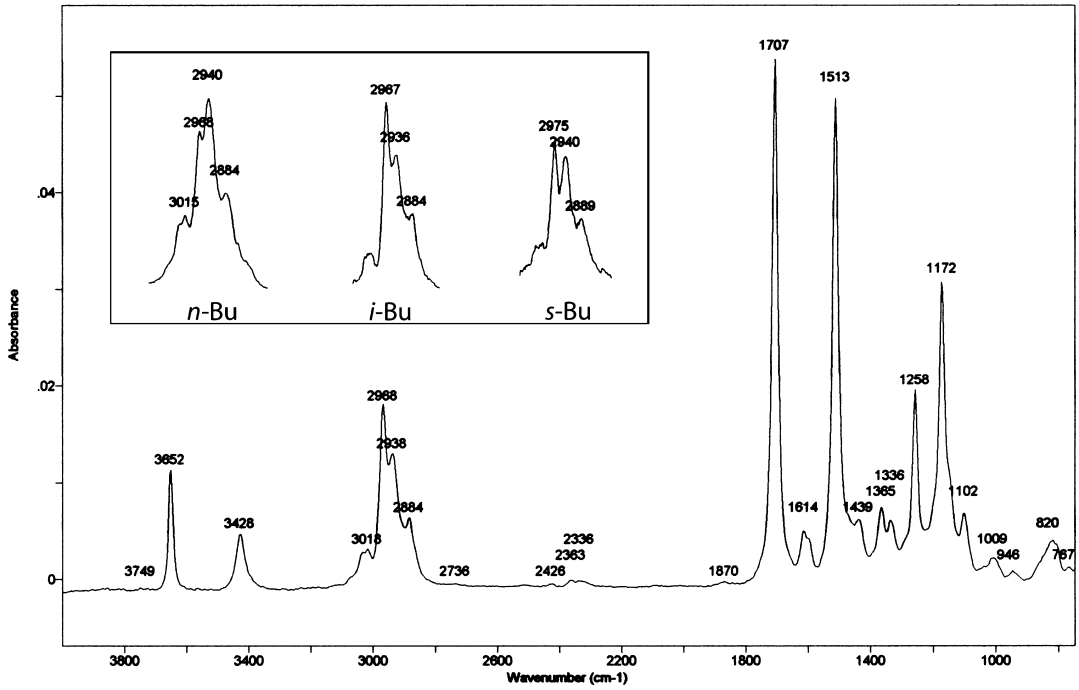


Figure 1. FTIR vapor-phase spectrum of the natural α-ketotyramide from males of the ant *M. minimum*. Insets are of the ν_{CH} region of synthetic **4a**, **4c**, and **4b** from left to right.

flight, an activity that may imply the presence of an attractant or flight-initiating signal emitted by the male ants.[24] Finally, it should be noted that the *Solenopsis* males that were available for this investigation are members of the subgenus *Diplorhoptrum*, commonly known as "thief ants", a group whose workers and queens contain a wide variety of venom alkaloids.[1,6] These ants are mostly subterranean, with extremely difficult taxonomy, and very little is known about their mating behavior.[25] Collections 11 and 78 from Puerto Rico may be *Solenopsis azteca*, *Solenopsis pygmaea*, or *Solenopsis torresi*, previously described species of thief ants found in the same region.[25] Because the mating flights of congeneric ant species have been observed to be separated spatially and temporally,[26] the production of the same tyramide (**3**) in the thief ant males as that in *S. maboya* may not be problematic if it were part of their mating behavior. More research is needed to clarify the role of these unusual and uncommon tyramides in the natural history of myrmicine ants, in particular to confirm a role in mating.

Experimental Section

General Experimental Procedures. GC−MS was carried out in the electron impact (EI) mode using a Shimadzu QP-5000 GC−MS equipped with an RTX-5, 30 m × 0.25 mm i.d. column. The instrument was programmed from 60 to 250 °C at 10 °C/min. Vapor-phase FTIR spectra were obtained using a Hewlett-Packard model 5965B detector interfaced with a Hewlett-Packard 5890 gas chromatograph fitted with a 30 m × 0.25 mm RTX-5 amine column and a temperature program from 100 to 280 °C at 10 °C/min or occasionally slower ramps. Some work used a Phenomenex "Inferno" column (30 m × 0.22 mm). NMR spectroscopy was carried out in $CDCl_3$ solutions using a Varian Mercury 400 NMR spectrometer. HRMS was performed on a JEOL SX102 instrument in the positive-ion fast-atom bombardment mode using a direct probe and a Waters LCT Premier time-of-flight instrument in the electrospray ionization (ESI) or APCI mode.

Ants. Two collections of *M. minimum* Buckley males were collected in July 1995 and again in July 1996 in Lexington, VA. In each collection, 10−20 individuals were obtained and placed in vials containing a small amount of methanol for analysis. One of the 1996 collections was frozen while still alive; the frozen ants were trisected, and their heads, thoraxes, and gasters were placed in separate vials containing a small amount of methanol. A collection of *M. ebinenum* Forel males was made in Guaynabo, Puerto Rico (PR), in June 1996 and another in July 1996, and a third collection was made in July 2003 in Cayey, PR. One of the 1996 collections of *M. ebeninum* was trisected, and their heads, thoraxes, and gasters were placed in separate vials containing a small amount of methanol. *M. floricola* Jerdon males were collected in May 1999 in Guaynabo, PR. *M. pharaonis* L. males were collected in July 1996 in Gainesville, FL, and these ants were placed in vials containing a small amount of methylene chloride for analysis. *M. opaciventris* workers, males, and queens were collected in March 2003 in Kakamega District, Isecheno, Kakamega Forest, Kenya, and placed in vials containing methanol. *Solenopsis* nr. *molesta* were collected in May 1993 in the Chuckawalla Mountains, Red Cloud Canyon, 8 mi. south-southeast of Desert Center, Riverside County, CA. *S. maboya* Snelling were collected in Guaynabo, PR, in February 1999 and January 2000, *Solenopsis* 11 males were collected in May 1995 near Guaynabo, PR, and *Solenopsis* 78 males were collected in June 1998 near Guaynabo, PR. The distribution of tyramides found in these species is shown in Table 1. Voucher specimens have been deposited in the entomological collection of the Los Angeles County Natural History Museum.

***N*-[2-(4-Hydroxyphenyl)ethyl]-2-hydroxyhexanamide (5a).** A solution containing 0.26 g of 2-hydroxyhexanoic acid[14] (2 mmol), 0.26 g of tyramine, 0.27 g of 1-hydroxybenzotriazole (HOBT), and 0.4 g of *N*-ethyl-*N*-[3-(dimethylamino)propyl]carbodiimide (EDCI) in 8 mL of DMF was stirred overnight at room temperature. The solvent was removed under reduced pressure, and the residue was partitioned between 2% HCl and diethyl ether. The ether layer was dried over anhydrous $MgSO_4$. After filtration, the solvent was removed to provide 0.55 g of **5a**: ^{13}C NMR (100 MHz, DMSO-d_6) δ 174.54, 156.31, 130.08 (2C), 130.05, 115.76 (2C), 71.61, 35.11, 34.74, 27.46, 22.74, 14.57 (the *C*H_2NH signal was obscured by the solvent signal); EIMS [M^+] *m*/*z* 251 (1), 194 (1), 164 (1), 132 (4), 121 (17), 120 (100), 107 (12), 91 (3), 77 (6), 69 (5); HRMS *m*/*z* 251.1570, calcd for $C_{14}H_{21}NO_3$, *m*/*z* 251.1521.

***N*-[2-(4-Hydroxyphenyl)ethyl]-2-hydroxy-3-methylpentanamide (5b).** A solution containing 0.26 g of 2-hydroxy-3-methylpentanoic acid[14] (2 mmol) was condensed with tyramine as described for **5a** to provide 0.8 g of a mixture that was 70% pure, with the remainder being benzotriazole: ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.29 (5H, m), 4.48 (1H, t, J = 5.2 Hz), 4.09 (2H, dd, J = 11.6 and 5 Hz), 3.75 (4H, m), 2.78 (2H, m), 2.60 (1H, m), 2.07 (1H, m), 1.8−1.4 (13H, br m); ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.77, 156.27, 130.11 (2C), 130.08, 115.74 (2C), 75.72, 38.72, 35.11, 23.69, 16.31, 12.44 (the *C*H_2NH signal was obscured by the solvent signal); EIMS [M^+] *m*/*z* 251 (1), 194 (2),

164 (2), 132 (6), 121 (17), 120 (100), 107 (11), 91 (4), 77 (8), 45 (9); HRMS *m/z* 251.1543, calcd for $C_{14}H_{21}NO_3$, *m/z* 251.1521.

***N*-[2-(4-Hydroxyphenyl)ethyl]-2-hydroxy-4-methylpentanamide (5c).** A solution containing 0.26 g of 2-hydroxy-4-methylpentanoic acid[14] (2 mmol) was condensed with tyramine as described for **5a**: ^{13}C NMR (100 MHz, DMSO-d_6) δ 174.93, 156.28, 130.14 (2C), 130.1, 115.75 (2C), 70.22, 44.17, 35.08, 24.54, 24.12, 22.20 (the *C*H_2NH signal was obscured by the solvent signal); EIMS [M^+] *m/z* 251 (1), 194 (1), 164 (2), 132 (3), 121 (16), 120 (100), 107 (13), 91 (3), 77 (6), 45 (1), 43 (6); HRMS *m/z* 251.1521, calcd for $C_{14}H_{21}NO_3$, *m/z* 251.1521.

***N*-[2-(4-Hydroxyphenyl)ethyl]-2-oxohexanamide (4a).** A solution containing 60 mg of **5a** in 3 mL of toluene and 20 mg of V_2O_5 was heated at 100 °C overnight. The cooled solution was filtered through a short column of Florisil to provide 45 mg of **4a** that was more than 75% pure by GC−MS analysis. No starting material remained: ^{1}H NMR of the crude material (400 MHz, $CDCl_3$) δ 6.93 (2H, d, J = 8.2 Hz), 6.74 (2H, d, J = 8.2 Hz), 3.43 (2H, q, J = 5 Hz), 3.41 (1H, m), 2.83 (2H, t, J = 7 Hz), 2.68 (2H, t, J = 6.8 Hz), 2.10 (1H, s), 1.50 (2H, quintet, J = 7 Hz), 1.25 (2H, sextet, J = 7 Hz), 0.82 (3H, t, J = 7.1 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 198.18, 159.17, 153.71, 128.84 (2C), 128.84, 114.63 (2C), 39.72, 35.44, 33.49, 24.20, 21.15, 12.79; EIMS [M^+] *m/z* 249 (1), 164 (5), 121 (30), 120 (100), 107 (25), 91 (6), 85 (9), 77 (13), 57 (20); HRCIMS ([M + H]$^+$) *m/z* 250.1445, calcd for $C_{14}H_{20}NO_3$, *m/z* 250.1443.

***N*-[2-(4-Hydroxyphenyl)ethyl]-3-methyl-2-oxopentanamide (4b).** A solution containing 0.5 g (2 mmol) of crude **5b** in 20 mL of acetone was cooled to 0 °C and treated with 2 mL of the Jones reagent. After 15 min, 3 mL of 2-propanol were added, and the solution was taken up in 50 mL of ether. The solution was dried over anhydrous $MgSO_4$ and filtered, and the solvent was removed under reduced pressure. Flash chromatography with silica gel (hexane/ethyl acetate) provided 60 mg of **4b** for spectroscopic analysis: ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.97 (2H, d, J = 8.2 Hz), 6.72 (2H, d, J = 8.2 Hz), 3.44 (2H, q, J = 5 Hz), 3.41, (1H, m), 2.70 (2H, d, J = 7 Hz), 2.7 (2H, J = 6.8 Hz), 2.08 (1H, m), 1.01 (2H, d, J = 7 Hz), 0.81 (3H, t, J = 7.1 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 201.19, 159.08, 153.90, 128.75 (2C), 128.67, 114.63 (2C), 39.82, 39.37, 33.50, 24.38, 14.07, 10.45; EIMS [M^+] *m/z* 249 (1), 164 (7), 121 (42), 120 (100), 107 (16), 91 (6), 85 (10), 77 (11), 57 (50); HRCIMS ([M + H]$^+$) *m/z* 250.1442, calcd for $C_{14}H_{20}NO_3$, *m/z* 250.1443.

***N*-[2-(4-Hydroxyphenyl)ethyl]-4-methyl-2-oxopentanamide (4c).** A solution with the same amounts of reactants (but with **5c** instead of **5a**) as those described for **4a** provided 38 mg of **4c** after flash chromatography: ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.97 (2H, d, J = 8.2 Hz), 6.72 (2H, d, J = 8.2 Hz), 3.44 (2H, q, J = 5 Hz), 5.70 (2H, br m), 2.70 (2H, t, J = 6.8 Hz), 2.08 (1H, m), 0.86 (6H, d, J = 6.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 199.06, 160.48, 154.83, 130.24, 130.03 (2C), 115.84 (2C), 45.48, 40.96, 34.75, 24.58, 22.75 (2C); EIMS [M^+] *m/z* 249 (3), 164 (7), 121 (37), 120 (100), 107 (20), 91 (3), 85 (9), 77 (9), 57 (25), 41 (8); HRCIMS ([M + H]$^+$) *m/z* 250.1443, calcd for $C_{14}H_{20}NO_3$, *m/z* 250.1443.

The GC retention time and MS and FTIR spectra of **4c** were identical with those of the α-ketotyramides found in *M. minimum*, *M. ebeninum*, and *M. pharaonis*.

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