of isomers 10 that, on reaction with DBN as described for the preparation of 4, afforded racemic 3: 113 mg (overall 9%, reaction conditions not optimized); mp 54-55 °C (ethyl ether/petroleum ether); ^{1}H NMR ($CDCl_3$) δ 2.67 (m d, 1 H, J = 19.8 Hz), 2.71 (br s, 1 H), 2.95 (d, 1 H, J = 19.8 Hz), 3.30 (br s, 1 H), 3.44 (br s, 1 H), 3.76 (s, 3 H), 4.64 (br s, 1 H), 6.80 (br s, 1 H).

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Registry No. (-)-1, 40983-58-2; (±)-3, 76947-23-4; (-)-4, 76985-84-7; (±)-4, 76985-85-8; 5, 77026-72-3; 6, 76947-24-5; (±)-8, 76947-25-6; (±)-9 (isomer 1), 76947-26-7; (±)-9 (isomer 2), 76985-86-9; (±)-10 (isomer 1), 76985-87-0; (±)-10 (isomer 2), 76985-88-1; dimethyl azodicarboxylate, 2446-84-6; bis(carbomethoxy)hydrazine, 17643-54-8; diethyl azodicarboxylate, 1972-28-7; bis(carboethoxy)hydrazine, 4114-28-7.

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3-Oxo-(*Z*)-9-hexadecenal: An Unusual Enolic β-Keto Aldehyde from a Termite Soldier Defense Secretion

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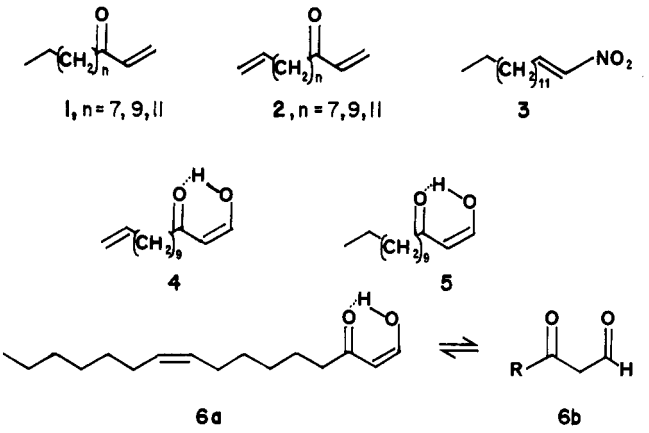
Chemical defense by soldier termites of the advanced subfamilies Prorhinotermitinae and Rhinotermitinae (Isoptera, Rhinotermitidae) is effected by the topical application of large quantities of a lipophilic contact insecticide to the surface of an attacking arthropod.[2,3] The primary evolutionary trend in soldier morphology in the subfamily Rhinotermitinae is the development of a minor soldier caste with reduced mandibles, an elongated labrum (the "upper lip") used as a paint brush for dispensing the contact poison, and the hypertrophy of the cephalic frontal gland to include a voluminous abdominal reservoir.[4] We have proposed[5] that the evolution of the defense chemistry of these termites has resulted in the production of increasingly reactive electrophiles in the more advanced genera. Thus, vinyl ketones (e.g., **1**),[6] α,ω-dienones (e.g., **2**),[7] and nitroolefin **3**[8] (Chart I) have been found in termites along this phyletic line. In addition, our recent discovery of the highly reactive enolized β-keto aldehydes **4** and **5** in *Rhinotermes hispidus*[5] supports this hypothesis. We

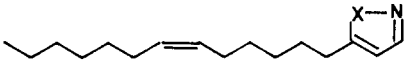
Chart I. Defense Secretions of Rhinotermitidae



now report that the most advanced termite of this subfamily, *Acorhinotermes subfusciceps*, possesses the 16-carbon β-keto aldehyde **6** as the major component of its defense secretion.

Compound **6** was obtained by hexane extraction of whole minor soldiers, and the presence of doublets (J = 4.3 Hz) at δ 7.92 (H-1a) and 5.52 (H-2a) in the ^{1}H NMR signaled the presence of a long-chain enolic β-keto aldehyde.[5] The dicarbonyl form was also present (<10%), showing ^{1}H resonances at δ 9.69 (t, J = 3 Hz, H-1b) and 3.44 (d, J = 3 Hz, H-2b). The nature of the long chain was suggested by the absorption of vinylic protons (δ 5.34, t, J = 5.3 Hz, H-9,10), the methylene protons adjacent to the C-3 ketone (δ 2.34, t, J = 7.2 Hz, H-4), four allylic methylene protons (δ 2.0, br m, H-8, 11), and a terminal methyl group (δ 0.88, t, J = 5 Hz, H-16). Gas chromatography–electron-impact mass spectroscopy of this crude secretion showed a single (>85%) major component [m/z (relative intensity) 252 (M^+, 1), 234 ($M^+ - H_2O$, 2), 86 ($\cdot H_2CC(^+OH)CH{=}CH_2$, 90), 71 ($H_2C{=}CHC{\equiv}O^+$, 100)] consistent with an unsaturated 16-carbon-chain β-keto aldehyde ($C_{16}H_{28}O_2$). The major fragments at m/z 86 and 71 can be attributed to the McLafferty-type rearrangement and α cleavage, respectively, both of which are known to occur in analogous substances.[5,7]

The unstable[9] β-keto aldehyde was not chromatographed but was converted to the pyrazole **7** by treatment with



7, X = NH
8, X = O

hdyrazine in ethanolic sodium hydroxide and to the isoxazole **8** by condensation with hydroxylamine in ethanol with potassium carbonate as the base. The pyrazole derivatives have the advantage of reasonably strong parent peaks in the mass spectra; however, they exist as slowly equilibrating tautomers which give broad GC peaks and broadened ^{1}H and ^{13}C NMR reasonances. Gas chromatographic–high-resolution mass spectroscopic analysis of pyrazole **7** showed m/z 248.2223 (4.7%) (calcd for $C_{16}H_{28}N_2$, 248.2252), confirming the presence of a tridecenyl side chain on a pyrazole nucleus. The isoxazoles are formed exclusively as the 5-substituted isomers as shown, and give sharp GC peaks and NMR resonances. In this case, it was clear that a single β-keto aldehyde was present, with a single isoxazole evident by capillary GC.

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(3) Quennedey, A. *La Recherche* **1975**, *6*, 274-277.
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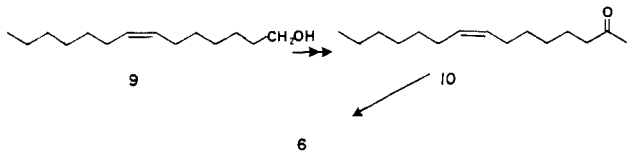
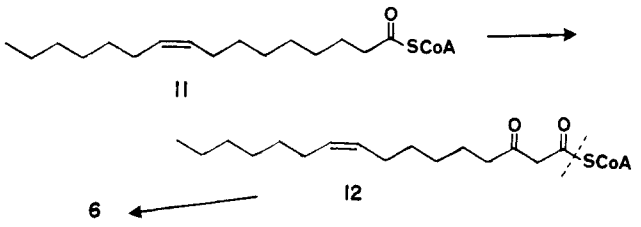
The location and geometry of the double bond was initially hypothesized to be (*Z*)-9 on the basis of biosynthetic considerations as supported by ^1H and ^{13}C NMR evidence. The ^1H NMR of pyrazole **7** derived from the termite β-keto aldehyde showed four allylic protons and two vinylic protons (δ 5.34, t, 5 Hz), suggesting an internal olefinic bond. ^{13}C NMR resonances for C-9 and C-10 at 129.88 and 130.14 ppm are consistent with similar shifts for (*Z*)-9-hexadecenoic acid derivatives;[10] these are upfield and more closely spaced than resonances in the *E* isomers.[11] We suspected that the biosynthetic origin for this compound (see below) was via desaturation followed by β oxidation of palmitic acid. In this case, 9,10-dehydrogenation to the *Z* isomer would be expected.

The structure of **6** was confirmed by total synthesis. Thus, a sample of (*Z*)-7-tetradecen-1-ol (**9**) was oxidized



to the corresponding aldehyde (pyridinium chlorochromate, CH_2Cl_2) and then converted to the methyl ketone **10** by methyllithium addition followed by Jones oxidation.[5] Formylation[5,12] then afforded the enolic compound **6** (25% overall from **9**) which was converted to pyrazole and isoxazole derivaitves. Spectral (NMR and mass spectra) and glass capillary chromatographic comparison of these substances with the corresponding termite-derived materials unambiguously established structure **6** for the natural material. The discovery of **6** as the defense secretion of a termite implicates a modification of the β oxidation of palmitic acid as a reasonable biogenetic pathway. Desaturation of palmitoyl coenzyme A to palmitoleoyl coenzyme A (**11**) could be followed by the



first three steps of the normal β-oxidation sequence for fatty acid degradation (desaturation, hydration, oxidation). The intermediate β-keto thioester **12** could undergo reductive cleavage as shown to provide β-keto aldehyde **6** or could suffer reductive C–C cleavage to give pentadecenone **10** which is also present in the secretion.[13] We are currently exploring the biogenesis and detoxication of these compounds in termites in vivo.

Experimental Section

NMR spectra were obtained on a Varian Associates CFT-20 NMR spectrometer operating at 80 MHz for ^1H and 20 MHz for ^{13}C. Shifts are reported for deuteriochloroform solutions in parts per million downfield from $(CH_3)_4Si$ by using the $CHCl_3$ resonance as the internal standard for ^1H NMR and the $CDCl_3$ resonance for ^{13}C NMR. Microprobe ^{13}C NMRs of **7** and **8** were performed in C_6D_6 by using a 1.7-mm capillary tube or in a 200-μL, 8-mm-diameter microcell. Gas chromatography was performed on a Varian Model 3700 gas chromatograph equipped with a 19 m × 0.5 mm glass capillary coated with OV-101 and operating at 120–200 °C at 12 °C/min. Low-resolution mass spectra were obtained on a Hewlett-Packard HP5980A mass spectrometer interfaced to an HP 5710A gas chromatograph GC equipped with a 2 m × 2 mm i.d. glass column packed with 3% OV-17 on 100/120 Gas Chrom Q. High-resolution mass spectra were performed on an MS-30 instrument interfaced to a gas chromatograph and a DS-50 data system. Solvents were Fisher HPLC grade and were used without further purification.

Isolation. Colonies of *Acorhinotermes subfusciceps* (Emerson) were obtained from rotten woody litter in the rainforest at Kartabo, Guyana, and were hand carried to Stony Brook. Previous experience had shown that removal of the soldiers in the field and extraction on-site resulted in complete decomposition of the unstable keto aldehydes prior to chemical analysis. Therefore, living soldiers were removed from the colony, frozen (1 h) and crushed in hexanes; the crude extract was then filtered through glass wool. Whole soldiers were used for extraction of the defense secretion since the cephalic gland is known to have extensive abdominal reservoirs. Approximately 10 mg of crude secretion was obtained from 130 soldiers. The crude extract was concentrated in vacuo and used directly for NMR (see text) and mass spectroscopy (see text) and chemical derivatization.

Pyrazole 7. A 4-mg sample of the crude secretion was dissolved in 0.3 mL of absolute ethanol and cooled to 0 °C with stirring while 4 drops of 2 N NaOH and 4 drops of anhydrous hydrazine were added. The reaction was allowed to stir from 0–20 °C for 3 h. After the mixture was quenched with 2 N HCl and water, the pyrazole was extracted with hexane–ether (1:1) and purified by flash chromatography in a Pasteur pipet by successive elution with 10% and 25% ethyl acetate–hexane mixtures. Spectral data were identical with those presented below for synthetic **7**.

Isoxazole 8. A 3-mg sample of crude secretion was dissolved in 0.2 mL of absolute ethanol and treated at 0 °C with ca. 20 mg of dry K_2CO_3 and ca. 20 mg of hydroxylamine hydrochloride. The reaction was stirred at 0–20 °C for 1 h and at 80 °C for 2 h. Isolation was similar to that for the pyrazole, except the isoxazole eluted with 5% ethyl acetate–hexane. Spectral data were identical with those of synthetic **8** presented below.

(*Z*)-8-Pentadecen-2-one (10). A solution of (*Z*)-7-tetradecen-1-ol [330 mg from hydrolysis (K_2CO_3, C_2H_5OH, 16 h) of the acetate **9** obtained from Farchan Chemicals] in 5 mL of CH_2Cl_2 was added in one portion to a suspension of 500 mg of pyridium chlorochromate in 10 mL of CH_2Cl_2. The mixture was stirred 3 h at 20 °C, diluted with ether, filtered through Florisil, and concentrated in vacuo. The crude aldehyde (300 mg) was dissolved in 10 mL of dry THF and treated with 2.0 mL of 1.8 M ethereal methyllithium. After the mixture was stirred 2 h at 0–20 °C, the reaction was quenched and worked up in a usual fashion to give the crude methylcarbinol. The crude carbinol in acetone solution was treated with a slight excess of Jones reagent, a few drops of 2-propanol were added, and the mixture was diluted with hexane and filtered through Florisil to give crude methyl ketone **10**. Flash chromatography (5% ethyl acetate–hexane) afforded 215 mg of the GC and TLC homogenous ketone: ^1H NMR ($CDCl_3$) δ 5.33 (br t, J = 5.4 Hz, H-8,9), 2.41 (t, J = 7.1 Hz, H-3), 2.12 (s, H-1), 2.08–2.0 (m, H-7, 10), 0.87 (br t, J = 4.9 Hz); ^{13}C NMR ($CDCl_3$) δ 208.75 (C-2), 130.10, 129.41 (C-8,9) 43.63 (C-3), 31.74 (C-13), 29.68, 29.47 (×2), 28.94, 28.77, 27.18, 26.96 (C-4–7,10–12), 23.70 (C-1), 22.61 (C-14), 14.00 (C-15).

3-Oxo-(*Z*)-9-hexadecenal (6). A solution of 192 mg (0.85 mmol) of **10** and 0.15 mL of ethyl formate in 5 mL of dry benzene was added dropwise to a stirred suspension of 25 mg of finely chopped sodium metal in 5 mL of dry benzene.[12] The mixture was stirred 1 h at room temperature and then 1 h at reflux. The cooled reaction mixture was decanted into water in a separatory funnel, and the flask containing unreacted sodium was rinsed with ether. The layers were separated, and the organic layer was washed with 2 N NaOH. The combined basic aqueous layers were washed with 1:1 ether–hexane and acidified to pH 3–4 with 4% HCl, and β-keto aldehyde **6** was extracted with ether–hexane. No attempts were made to further purify the chromatographically unstable β-keto aldehyde:[9] 95 mg (44%); ^1H NMR ($CDCl_3$) δ 9.81

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(t, J = 1.6 Hz, H-1b), 7.92 (d, J = 4.3 Hz, H-1a), 5.52 (d, J = 4.3 Hz, H-2a), 5.34 (br t, J = 5.4 Hz, H-9,10), 3.50 (d, J = 1.6 Hz, H-2b), 2.33 (t, J = 6.7 Hz, H-4), 0.88 (br t, J = 4.9 Hz, H-16); ^{13}C NMR ($CDCl_3$) δ 199.75 (s, C-3), 175.77 (d, C-1a), 130.35, 129.48 (d, d, C-9,10), 101.79 (d, C-2a), 39.55 (t, C-4), 31.85 (t, C-13), 29.78, 29.50, 29.06, 28.90, 27.30, 27.04, 25.21 (C-5–8,11,12), 22.71 (t, C-14) 14.14 (q, C-15).

Pyrazole and isoxazole derivatives were prepared as described above. Spectral data for isoxazole 8: ^{1}H NMR ($CDCl_3$) δ 8.12 (d, J = 1.7 Hz, H-1), 5.95 (d, J = 1.7 Hz, H-2), 5.34 (br t, J = 5.5 Hz), 2.77 (t, J = 7.7 Hz, H-4), 2.04–1.98 (m, H-8, H-11), 0.88 (br t, J = 5 Hz, H-16); ^{13}C NMR (C_6D_6) δ 172.51 (C-3), 149.71 (C-1), 130.29, 129.63 (C-9,10), 99.53 (C-2), 32.01 (C-14), 29.98, 29.45, 29.22, 28.72, 27.52 (×2), 27.21, 26.38 (C-4–8,11–13), 22.89 (C-15), 14.14 (C-16); electron-impact mass spectrum (70 eV), m/z (relative intensity) 249 (1), 232 (2), 220 (4), 206 (6), 192 (7), 178 (7), 96 (100), 83 (41), 70 (52), 55 (60), 41 (42).

Spectral data for pyrazole 7: ^{1}H NMR ($CDCl_3$) δ 7.47 (br d, $J \simeq 1$ Hz, H-1), 6.09 (br d, $J \simeq 1$ Hz, H-2), 5.34 (br t, J = 5.4 Hz, H-9,10), 2.67 (br t, $J \simeq 7.2$ Hz, H-4) 2.04–1.98 (br m, H-8,11), 0.88 (br t, $J \simeq 5.5$ Hz); ^{13}C NMR (C_6D_6) δ 147.82 (C-3), 134.70 (C-1), 130.14, 129.87 (C-9,10), 103.45 (C-2), 32.02 (C-14), 29.99, 29.72 (×2), 29.19 (×2), 27.49, 27.42, 26.95 (C-4–8,11–13), 22.89 (C-15), 14.14 (C-16); the resonances at δ 147.82, 134.70, and 103.45 were severely broadened due to the presence of both pyrazole tautomers; electron-impact mass spectrum (70 eV), m/z (relative intensity) 248 (3, M^+), 219 (4), 205 (19), 191 (13), 177 (17), 163 (12), 149 (16), 95 (100), 82 (95), 81 (66), 40 (38). The largest peaks are attributed to α cleavage and McLafferty-type rearrangement.[5]

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Registry No. 6, 76963-23-0; 7, 76963-24-1; 8, 76963-25-2; 9, 40642-43-1; 10, 76963-26-3.

Proton and Carbon-13 Nuclear Magnetic Resonance Spectroscopy and Conformational Aspects of the Curine Class of Bis(benzylisoquinoline) Alkaloids

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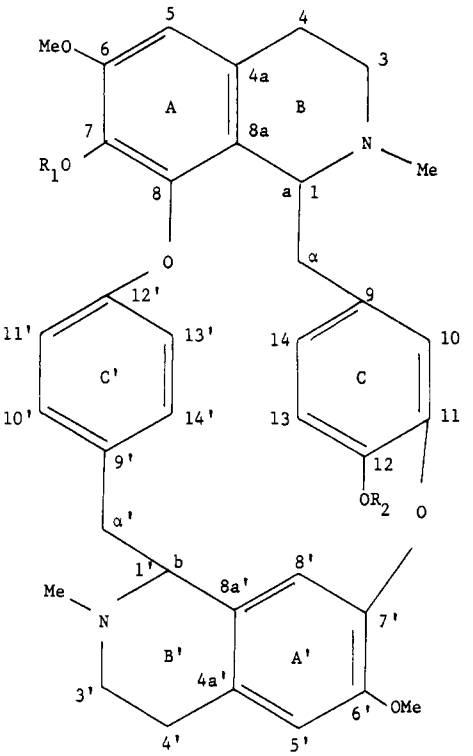
Due to the neuromuscular blocking activity shown by some members of the curine class of bis(benzylisoquinoline) alkaloids[1] much effort has been devoted to the elucidation of their structures, and more recently, by the application of modern spectroscopic techniques, data on their solution conformations have also been reported.[2]

The increasing use of ^{13}C NMR spectroscopy for structure elucidation of natural products and the lack of data on the bis(benzylisoquinolines) prompted us to analyze different classes of these alkaloids[3,4] in the hope of contributing to the determination of related but up to now unresolved structures[5] as well as to the detection, in combination with ^{1}H NMR, of conformational features of these interesting substances.

The ^{13}C NMR analyses were initiated with bebeerine (1) and its derivatives **2–7**.



1. $R_1 = R_2 = H$ (a,b = R,R)
2. $R_1 = R_2 = Me$ (a,b = R,R)
3. $R_1 = Me$, $R_2 = H$ (a,b = R,R)
4. $R_1 = H$, $R_2 = Me$ (a,b = R,R)
5. $R_1 = Me$, $R_2 = Ac$ (a,b = R,R)
6. $R_1 = Ac$, $R_2 = Me$ (a,b = R,R)
7. $R_1 = Ac$, $R_2 = Ac$ (a,b = R,R)
8. $R_1 = R_2 = H$ (a,b = R,S)

Table I lists their carbon shifts which were assigned by standard chemical shift theory and analysis of the SFORD and fully coupled spectra. Due to the overlapping of signals, the application of inversion recovery conditions was necessary to detect the sp^2 carbons nonbonded to hydrogen. The assignment was further supported by the known effects of alkylation and acetylation of phenols, analysis of model compounds, and selective irradiations.

The carbons of rings ABC and A′B′C′ were assigned, taking isochondodendrine (**9**) and 1-(*p*-methoxybenzyl)-6,7-dimethoxytetrahydroisoquinoline (**10**)[3] as models, respectively. The assignments of rings A and C′ carbons of **1** were in accordance with rings A and C of **9**, respectively, showing the same C–H nonequivalence in ring C as well. Confirmation of ring A chemical shifts was carried out by methylation, going from **1** to **2**, **3**, and/or **5**, and acetylation, going from **1** to **6** and/or **7**, of the phenols.[4–6] The

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