of three modified amino acid residues containing an α,β-unsaturated amide group conjugated with an oxazole[12] as well as an α-keto-β-amino acid moiety constituting a homoleucine-like unit. Interpretation of the HMBC and NOESY spectral data provided evidences for the amino acid sequence of **1**. As a result, **1** was deduced to be composed of a cyclic moiety and a side chain. The cyclic moiety consisted of segment **4**, Pro, Orn, and BhTrp connected in this sequence. The side chain was attached to the α-NH of the Orn residue and consisted of nVal and Ile. The N-terminus of Ile was shown to be protected by the 2-hydroxy-3-methylpentanoic acid group (Hmp).[13] Further substantial evidence for the structure of keramamide B (**1**) was obtained by the FAB MS/MS[14] spectrum of the $(M + H)^+$ ion of **1** (m/z 1137), which afforded daughter ions corroborating well the amino acid sequence deduced from the NMR results. The chiral GC analysis (Chirasil-Val, Alltech) of the *N*-trifluoroacetyl/methyl ester derivatives of the hydrolysate of **1** revealed that all of the Aba,[15] Pro, Orn, nVal, and Ile residues of **1** bore the L configurations. The BhTrp residue in **1** was converted into aspartic acid (Asp) by ozonolysis of **1** followed by oxidation with CH_3CO_3H, while the C-11–N-14 moiety in segment **4** was transformed into leucine (Leu) by treatment with H_2O_2/NaOH. These Asp and Leu residues were determined to be L by the chiral GC method, revealing that the BhTrp and the β-amino amino acid in **4** were also L. Therefore, the structure of keramamide B was established to be that of **1**.

Molecular formulas of keramamides C (**2**) and D (**3**) were determined to be $C_{53}H_{75}O_{12}N_{10}Br$ and $C_{52}H_{73}O_{12}N_{10}Br$, respectively, by HRFABMS data. The amino acid analyses and FAB MS/MS results revealed that keramamide C (**2**) contained an Aba residue in place of the nVal residue in keramamide B (**1**). For keramamide D (**3**) the nVal in **1** was replaced by an Aba residue and the ethyl group attached to C-9 of segment **4** was substituted by a methyl group.[16]

Keramamides B–D (**1–3**; 5×10^{-8} M) inhibited the superoxide generation response of the human neutrophils[17] elicited with a chemotactic peptide, *N*-formyl-Met-Leu-Phe (fMLP), but did not inhibit that induced by phorbolmyristate acetate or immune complex.[18] The mechanism of action is currently under investigation.[19]

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Supplementary Material Available: Notes for spectral data and spectra of keramamides B–D (36 pages). Ordering information is given on any current masthead page.

(6) **1**: ^{1}H NMR (DMSO-d_6) [**4**] 6.58 (d, 15.0; 2), 7.15 (d, 15.0; 3), 8.21 (s; 5), 4.63 (m; 9), 9.04 (d, 5.6; NH-10), 4.89 (m; 13), 8.15 (d, 5.1; NH-14), [BhTrp] 8.08 (d, 10.0; NH), 4.60 (m; α), 2.85 (dd, 14.5, 2.8; β), 3.06 (dd, 14.5, 5.5; β'), 11.21 (s; NH-1'), 6.88 (d, 2; 4), 6.58 (dd, 8.7, 2; 6), 7.03 (d, 8.7, 7), 8.64 (s; OH-5), [Pro] 4.35 (br d, 3.9; α), 1.85 (m; β), 2.15 (m; β'), 3.51 (m; δ), 3.74 (m; δ'), [Orn] 7.83 (d, 7.8; α-NH), 4.49 (m; α), 3.35 (m; δ), 2.65 (m; δ'), 7.43 (m; δ-NH), [nVal] 8.05 (d, 8.1; NH), 4.25 (m; α), [Ile] 7.50 (d, 9.3; NH), 4.28 (m; α), 1.70 (m; β), [Hmp] 3.74 (m; α), 5.45 (m; OH-α), 1.70 (m; β), 0.90 (m; CH_3-γ), 1.15 (m; CH_2-γ), and 0.90 (m; CH_3-δ); ^{13}C NMR (DMSO-d_6) [**4**] 163.9 (1), 123.5 (2), 127.4 (3), 139.6 (4), 136.9 (5), 164.8 (7), 50.0 (9), 160.1 (11), 195.8 (12), 52.5 (13), 39.0 (15), 24.8 (16), 20.8 (17), 23.2 (18), 25.2 (19), 10.5 (20), [BhTrp] 170.7 (CO), 53.4 (α), 27.9 (β), 109.5 (2'), 109.0 (3'), 102.3 (4'), 150.8 (5'), 111.5 (6'), 110.9 (7'), 130.5 (8'), 128.3 (9'), [Pro] 170.6 (CO), 58.4 (α), 29.6 (β), 23.2 (γ), 46.9 (δ), [Orn] 49.7 (α), 28.6 (β), 24.4 (γ), 38.9 (δ), [nVal] 171.2 (CO), 52.1 (α), 34.0 (β), 18.5 (γ), 13.6 (δ), [Ile] 169.4 (CO), 53.8 (α), 37.3 (β), 24.7 (γ), 15.4 (Me), 11.0 (Me'), [Hmp] 172.6 (CO), 75.0 (α), 38.2 (β), 24.2 (γ), 15.5 (Me), and 11.7 (Me').

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(9) Bax, A.; Summers, M. F. *J. Am. Chem. Soc.* **1986**, *108*, 2093–2094.

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(13) Carter, D. C.; Moore, R. E.; Mynderse, J. S.; Niemczura, W. P.; Todd, J. S. *J. Org. Chem.* **1984**, *49*, 236–241.

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(15) The Aba residue was derived from the C-7–N-10 moiety in segment **4**.

(16) Keramamides B–D (**1–3**) were shown to possess closely related structures to that of a peptide recently isolated by Professor N. Fusetani and his co-workers. The ^{1}H NMR and MS spectral data were compared with each other before submission of this manuscript.

(17) Shirato, M.; Takahashi, K.; Nagasawa, S.; Koyama, J. *FEBS Lett.* **1988**, *234*, 231–234.

(18) Human neutrophils are known to generate superoxide anion, when exposed to appropriate stimuli, such as phorbolmyristate acetate, ovalbumin complex of immunoglobulin G_2 antibody, and fMLP. Therefore the present results suggest that a certain factor inhibited by keramamides B–D (**1–3**) is involved in the intracellular signal transduction processes initiated by fMLP.

(19) Keramamides B–D (**1–3**) exhibited no cytotoxicity against murine lymphoma L1210 and human epidermoid carcinoma KB cells in vitro at 10 μg/mL.

New Synthesis of Methyl 1,3-Butadiene-2-carboxylate by the Cheletropic Extrusion of Carbon Monoxide from 3-Carbomethoxy-3,4-pentadienal and a Study of Its Dimerization To Give Dimethyl Mikanecate (Dimethyl 4-Ethenyl-1-cyclohexene-1,4-dicarboxylate)

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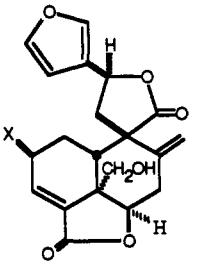
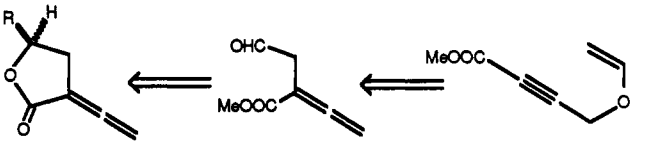
For a synthetic approach to plaunol B and C (**1a,b**), diterpenoids isolated from *Croton sublyratus* Kurz which show anti peptic ulcer activity,[1] we required an easy preparation of the 5-(3-furyl) allenic lactone **2a**. A reasonably short route would involve the thermal [3,3]-sigmatropic rearrangement[2] of the readily available 3-carbomethoxypropynyl ethenyl ether **3** to give the allenic acetaldehyde **4** followed by addition of an aryl anion and subsequent lactone formation. We report here the discovery of a novel thermal rearrangement of 3-carbomethoxy-3,4-pentadienal (**4**) to produce methyl 1,3-butadiene-2-carboxylate (**5**). In addition, we present the kinetic parameters for the very facile dimerization of **5** via a Diels–Alder cycloaddition.[3]



1a X = H
1b X = OH



2a R = 3-furyl
2b R = Ph

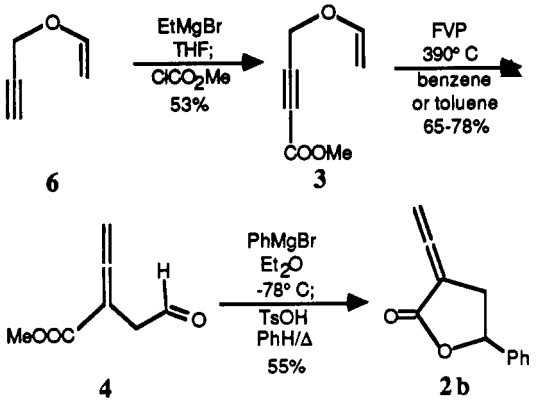
4 **3**

(1) Kitazawa, E.; Sato, A.; Takahashi, S.; Kuwano, H.; Ogiso, A. *Chem. Pharm. Bull.* **1980**, *28*, 227 and references therein.

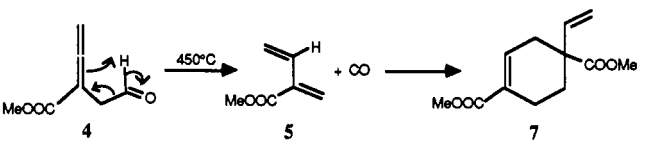
(2) (a) Black, D. K.; Landor, S. R. *J. Chem. Soc.* **1965**, 6784. (b) Black, D. K.; Fomum, Z. T.; Landor, P. D.; Landor, S. R. *J. Chem. Soc., Perkin Trans. 1* **1973**, 1349. (c) For a good recent review of allene rearrangements, see: Braverman, S. Rearrangements involving Allenes. In *The Chemistry of Double-bonded Functional Groups*; Patai, S., Ed.; Wiley: New York, 1989; Chapter 14.

0002-7863/91/1513-7813$02.50/0

Ester **3** was prepared from the readily available 2-propynyl ethenyl ether (**6**)[4] in 53% yield by formation of the anion (EtMgBr, THF, Δ) and trapping with methyl chloroformate. Flash vacuum pyrolysis of **3** was accomplished by introduction of a 0.5–1 M solution of **3** in benzene or toluene into a horizontal pyrolysis tube (i.d. = 1 cm) packed with glass helices heated to 390 °C at a vacuum of ~140 mm. Trapping the pyrolysate at −78 °C gave the desired 3-carbomethoxy-3,4-pentadienal (**4**) in 65–78% yield [^{1}H NMR ($CDCl_3$) δ 9.70 (1 H, t, J = 1.5 Hz), 5.26 (2 H, t, J = 2.2 Hz), 3.77 (s, 3 H), 3.31 (2 H, dt, J = 1.5, 2.2 Hz); ^{13}C NMR ($CDCl_3$) δ 214.8, 197.9, 166.7, 92.6, 79.7, 52.6, 42.9]. Contrary to reports in the literature,[2b] we did not observe any prototropic rearrangement of **4** to give the conjugated dienal; the unconjugated dienal **4** could be handled normally and even chromatographed without extensive decomposition. The preparation of allenic lactones such as **2** from **4** proved straightforward. For example, addition of phenylmagnesium bromide in ether at −78 °C to **4** followed by heating with catalytic tosic acid in benzene furnished the phenyl allenic lactone **2b** in 55% yield.[5] Thus this is a good approach to allenic lactones of this sort.



However, when the pyrolysis of **3** was carried out on a larger scale at a somewhat higher temperature, namely, ~450 °C (or the time that the compound was in the "hot zone", the so-called contact time, was increased), after a normal workup we obtained very little of the desired dienal **4**. The major product, isolated in 50–70% yield, was dimethyl mikanecate (**7**). Under more carefully controlled conditions (pyrolysis of a solution in benzene-d_6 or toluene-d_8 with trapping at −196 °C), it was easy to isolate the initial product of the further pyrolysis of **4**, namely, methyl 1,3-butadiene-2-carboxylate (**5**), and determine its structure by proton NMR [^{1}H NMR (toluene-d_8) δ 6.38 (1 H, dd, J = 17.6, 11.2 Hz), 6.02 (1 H, s), 5.71 (1 H, d, J = 17.6 Hz), 5.48 (1 H, s), 5.10 (1 H, d, J = 11.2 Hz), 3.41 (3 H, s)]. We propose that this reaction occurs via a thermally allowed six-electron cheletropic extrusion of CO.[6] As far as we can tell, there are no prior examples of this specific type of reaction although other similar thermal decarbonylations are known.[7] This turns out to be a fairly convenient way of preparing this very reactive dienic ester.



Although the high propensity of **5** toward Diels–Alder dimerization has been known for some time,[3] no good data are available on the kinetics of this process. We therefore carried out the dimerization of **5** to give **7** in toluene at several temperatures[8] and measured the rate of cyclization. The rate data was used in both the Eyring and Arrhenius equations to produce the following kinetic parameters (at 298 K):

$$E_a = 11.12 \pm 0.72 \text{ kcal/mol}$$

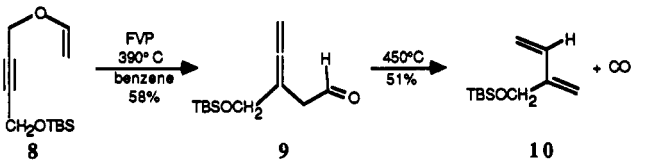
$$\Delta H^{\ddagger} = 10.67 \pm 0.95 \text{ kcal/mol}$$

$$\Delta S^{\ddagger} = -35.4 \pm 2.8 \text{ cal/(mol K)}$$

$$\Delta G^{\ddagger} = 21.22 \pm 1.9 \text{ kcal/mol}$$

Thus the dimerization of this diene has a lower activation energy than most other dienes. For example, the activation energy for the dimerization of cyclopentadiene at 20 °C in benzene is 16.4 kcal/mol.[9] The reasons behind this very fast dimerization of **5** are not totally clear, but probably involve a strong polarization of the 1,2-double bond, thus making it a very good dienophile (much better than the 1,2-double bond of methyl 1,3-butadiene-1-carboxylate, for example, which is reluctant to dimerize). In addition, the E (s-cis) conformation of the diene, necessary for the Diels–Alder reaction, is probably more stable than the Z (s-trans), thus making it easier to achieve the transition-state geometry. We intend to carry out further calculations on this system to try to determine in a more precise way the underlying reasons for the rapid dimerization.[10]

Finally we have also shown that this two-step procedure for the synthesis of 2-substituted butadienes is useful for the preparation of other dienes. Thus pyrolysis of the readily available 3-[(silyloxy)methyl]propargyl vinyl ether **8** gives the substituted pentadienal **9**, which can be further pyrolyzed at higher temperatures to give the 2-[(silyloxy)methyl]-1,3-butadiene **10** (yields not optimized). Further studies on the dimerization of **5** and the use of allenic lactones such as **2a,b** in the synthesis of the plaunols will be reported later.



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(5) The Diels–Alder cycloadditions of these allenic lactones **2** with various dienes, including those leading toward the plaunols, will be described separately. Jung, M. E.; Zimmerman, C. N.; Lowen, G. T., manuscript in preparation.

(6) Pyrolysis of **4** at atmospheric pressure and bubbling of the effluent through aqueous barium hydroxide produces barium carbonate and thus indicates that CO is lost in this reaction.

(7) For example, see: (a) Trahanovsky, W. S.; Emeis, S. L. *J. Am. Chem. Soc.* **1975**, *97*, 3773. (b) Trahanovsky, W. S.; Mullen, P. W. *J. Am. Chem. Soc.* **1972**, *94*, 5086.

(8) The NMR experiments were performed on a Bruker AM 360-MHz spectrometer equipped with a variable-temperature probe regulated to within ±1 °C of the desired temperature. The probe temperature was calibrated with an ethylene glycol standard. Spectra were obtained via an automated data acquisition program, which recorded spectra at prescribed time intervals. The relative concentrations in all experiments were calculated from the integrals of the appropriate peaks in the ^{1}H NMR spectra (δ 6.85 and 5.83 in **7** and δ 6.38, 6.02, and 5.48 in **5**), relative to an internal standard (1,3,5-trichlorobenzene).

(9) Sauer, J.; Sustmann, R. *Angew. Chem., Int. Ed. Engl.* **1980**, *19*, 779.

(10) We thank Mr. Iordanis Houdaverdis for carrying out some preliminary calculations on this system.