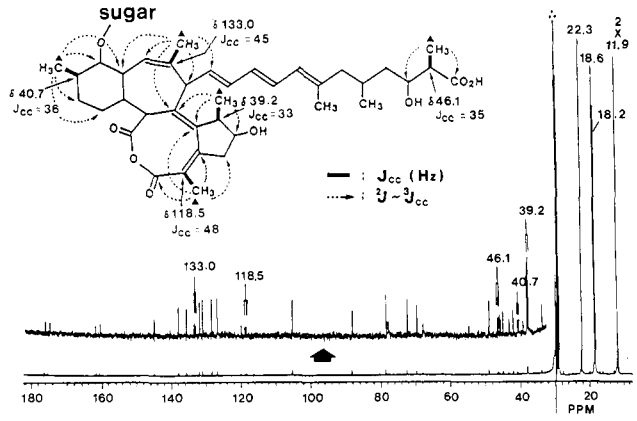


Figure 1. ^{13}C NMR spectrum and ^{13}C–^{13}C coupling pattern of [*methyl*-^{13}C]-L-methionine labeled aurantinin B.

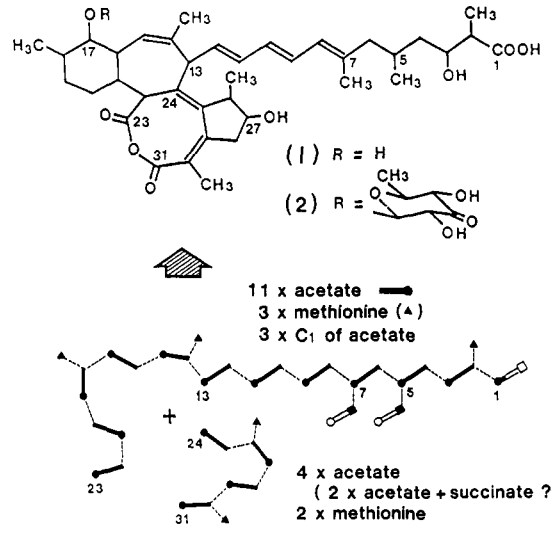


Figure 2. Structures and biosynthetic building units of aurantinins A (1) and B (2).

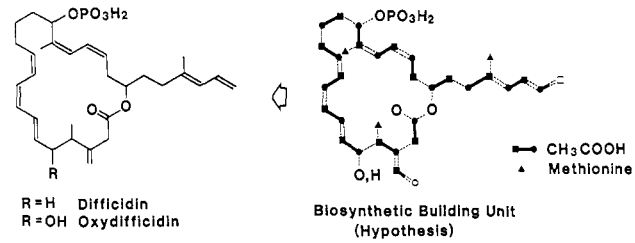
2 and the product of 2 by alkaline hydrolysis followed by methylation with CH_2N_2. Furthermore, the connectivity among structures A, B, and C was provided by ^{13}C spectral analysis of [methyl-^{13}C]-L-methionine labeled aurantinin B. The ^{13}C spectrum exhibited a high incorporation (90% or more) for five methyl carbons (δ 22.3, 18.6, 18.2, and 11.9 × 2). The location of these methyls was assigned to the tail position of acetate units in the polyketide chain because each carbon (δ 133.0, 118.5, 46.1, 40.7 and 39.2) attached directly to the five ^{13}C enriched methyl carbons appeared as a doublet. The observation of the C–C couplings from the enriched methyls to adjacent carbons from one to three bonds revealed the validity of a polycyclic structure for the aglycon of 2, as shown in Figure 1. This appears to be the first example in which high level incorporation by means of feeding experiments with [*methyl*-^{13}C]methionine was used effectively for structure determination of a microbial metabolite. It is noteworthy that 2 involves an acid anhydride moiety and a novel sugar, in addition to a polyketide skeleton containing four rings with five, six, seven, and eight members and a triene.

Antibiotics 1 and 2 seem to be built up biosynthetically from two polyketide chains. A long chain originates from 11 acetate units, three C_1 units arising from methionine, two C_1 units at C-5 and C-7 from acetate via decarboxylation, and one C_1 unit at C-1 from acetate being a starter unit. A short chain consists of four acetate units in which a succinate formed by "tail to tail condensation" of two acetate units might be the starter unit, and two C_1 units from methionine, as shown in Figure 2. The presence of a methyl group formed by decarboxylation of acetate seems to be common to true bacterial metabolites, as also reported in biosynthetic studies of myxopyronin.[6] The only exception to this is the virginiamycin family of antibiotics from actinomycetes.[7] Recently, Zimmerman et al. reported isolation and structure determination of the unusual macrolide antibiotics difficidin and oxydifficidin which were produced by a true bacterium, *Bacillus subtilis*.[8] Taking into consideration the findings detailed here for aurantinin biosynthesis, we can speculate that the tentative building units for difficidin consist of thirteen acetate units, three methionines, and two C_1 units arising from acetates.[8]

Acknowledgment. We are indebted to Professor T. J. Simpson, University of Edinburgh, for useful discussions.

Supplementary Material Available: Complete assignments of ^1H and ^{13}C chemical shifts in 400-MHz NMR are provided for compounds 1 and 2 (1 page). Ordering information is given on any current masthead page.

(6) Kohl, W.; Irschik, H.; Reichenbach, H.; Hofle, G. *Liebigs Ann. Chem.* **1984**, 1088–1093.
(7) (a) Kinston, D. G. I.; Kolpak, M. X.; Lefevre, J. W.; Grochtmann, I. B. *J. Am. Chem. Soc.* **1983**, *105*, 5106–5110. (b) Lefevre, J. W.; Kingston, D. G. *J. Org. Chem.* **1984**, *49*, 2588–2593.
(8) (a) Zimmerman, S. B.; Schwartz, C. D.; Monaghan, R. L.; Pelak, B. A.; Weissberger, B.; Gilfilan, E. C.; Mochales, S.; Hernandez, S.; Currie, S. A.; Tejera, E.; Stapley, E. O. *J. Antibiot.* **1987**, *40*, 1677–1681. (b) Wilson, K. E.; Flor, J. E.; Schwartz, R. E.; Joshua, H.; Smith, J. L.; Pelak, B. A.; Hensens, O. D. *J. Antibiot.* **1987**, *40*, 1682–1691.



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Synthesis of 2-Imidoglycolic Acids and a New Heterobifunctional Cross-Linking Agent, *N*-Succinimidyl 2-Maleimidoglycolate

Summary: The reaction of five-membered cyclic imides with glyoxylic acid produces the corresponding 2-imidoglycolic acids. The *N*-hydroxysuccinimide ester of 2-maleimidoglycolic acid is introduced as a new heterobifunctional cross-linking agent for protein modification.

Sir: The reaction of amides and carbamates with glyoxylic acid monohydrate in either acetone or diethyl ether is known to produce the corresponding 2-amido and 2-car-

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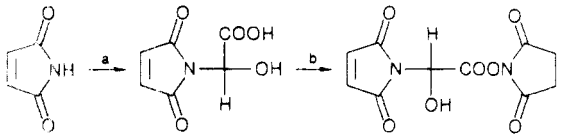
Scheme I[a]

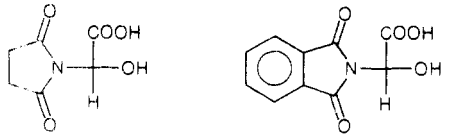


3 4

[a] Reagents: (a) glyoxylic acid·H_2O, 1.5 equivs, acetone, reflux, 90% based on recovered maleimide; (b) *N*-hydroxysuccinimide, DCC, DME, 4 °C, 51%.

bamato glycolic acids in moderate to good yields.[1] Described herein is an extension of this reaction to five-membered cyclic imides which allows for the preparation of 2-imidoglycolic acids as potential ligands for biologically interesting metals[2] and, in one case, as a precursor to a new heterobifunctional cross-linking agent, *N*-succinimidyl 2-maleimidoglycolate, MGS.

Succinimido, phthalimido, and maleimidoglycolic acids **1**, **2**, and **3**, respectively, are produced by the reaction of the corresponding cyclic imide with glyoxylic acid monohydrate in refluxing acetone. The former two are isolated as crystalline solids while the latter is afforded only as a heavy syrup. Characteristic to all three, however, is the presence of a clean singlet at 5.69–5.90 ppm in the ^1H NMR spectrum which is attributable to the α proton resonance of the product.[3]



1 2

Conversion of the syrupy 2-maleimidoglycolic acid to its crystalline *N*-hydroxysuccinimide ester **4** was easily achieved by the reaction of **3** with *N*-hydroxysuccinimide in the presence of DCC in DME at 4 °C (Scheme I).

Very much like *m*-maleimidobenzoyl *N*-hydroxysuccinimide ester, MBS,[4] in its ability to cross-link drugs, enzymes, etc., to proteins, MGS has the added potential for the pH-dependent controlled release of a drug or enzyme from the protein to which it is coupled. Precedent for this behavior can be found in a study by Bundgaard and Buur involving the pH-dependent hydrolysis of amidoglycolic acids and amidoglycolates.[5] Pseudo-first-order rate constants for decomposition with U-shaped pH-dependent curves indicative of both specific acid and base catalysis as well as a spontaneous or water-catalyzed reaction were observed, with minimal decomposition occurring at pH 3–4. In preliminary experiments designed to test the feasibility for controlled release, *p*-nitroaniline (pNA) was coupled to bovine albumin via MGS, utilizing methodology described by Kitagawa and co-workers,[6] and was found to release pNA gradually with time at physiological pH and temperature.[7] This characteristic is critical to therapy in which a controlled or prolonged release of a drug or enzyme into the serum or at the surface of a targeted cell is desired.

(1) Ben-Ishai, B.; et al. *Tetrahedron* **1976**, *32*, 1571–1573.
(2) For an interesting application of this, see: Serino, A. J. U.S. Pat. Appl. 084 037, August 11, 1987.
(3) All new compounds have been fully characterized by IR, ^1H NMR, and combustion analysis.
(4) (a) Kitagawa, T.; Aikawa, T. *J. Biochem.* **1976**, *79*, 233–236. (b) Kitagawa, T.; Fujitake, T.; Tamiyama, H.; Aikawa, T. *Ibid.* **1978**, *83*, 1493–1501.
(5) Bundgaard, H.; Burr, A. *Int. J. Pharm.* **1987**, *37*, 185–194.
(6) Kitagawa, T.; Kawasaki, T.; Munechika, H. *J. Biochem.* **1982**, *92*, 585–590.
(7) Details of this experimentation will be reported at a later date elsewhere.

Acknowledgment. Special thanks to Ms. Candace Keene for obtaining the combustion analyses.

Supplementary Material Available: Experimental procedures for the preparation of 1–4 and complete spectral data (3 pages). Ordering information is given on any current masthead page.

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1,7-Methanohomopentaprismane:[1] A [2.2.1]Propellane

Summary: The intramolecular cycloaddition of 11-methylene-8-pentacyclo[5.4.0.$0^{2,6}$.$0^{3,10}$.$0^{5,9}$]undecanylidene to the olefinic bond leads to 1,7-methanohomopentaprismane, a highly elusive [2.2.1]propellane, which spontaneously abstracts two hydrogen atoms from its environment, yielding 1,3-bishomopentaprismane.

Sir: We report strong evidence for the transient existence of 1,7-methanohomopentaprismane[1] (**6**) a [2.2.1]propellane. This is the first report of a [2.2.1]propellane being produced by the simultaneous formation of *two* carbon–carbon bonds.

Propellanes containing six or fewer bridge carbons have been of considerable recent interest, both experimental[2-10] and theoretical.[11] They possess two inverted carbon atoms and are highly reactive toward free radicals and electrophiles, but entirely inert toward nucleophiles. Such chemical behavior as well as recent experimental and theoretical studies indicates a significant electron density outside the inverted carbon atoms and, consequently, a reduced electron density between them compared with that between tetrahedral carbons. Whereas [1.1.1]-,[3]

(1) Heptacyclo[5.4.1.$0^{1,7}$.$0^{2,6}$.$0^{3,10}$.$0^{5,9}$.$0^{8,11}$]dodecane.
(2) Reviews: Wiberg, K. B. *Acc. Chem. Res.* **1984**, *17*, 379–386. Ginsburg, D. *Propellanes*; Verlag Chemie: Weinheim, 1975. Its sequels I and II, Department of Chemistry, Technion: Haifa, 1981 and 1985, respectively.
(3) (a) Wiberg, K. B.; Walker, F. H. *J. Am. Chem. Soc.* **1982**, *104*, 5239–5240. (b) Semmler, K.; Szeimies, G.; Belzner, J. *J. Am. Chem. Soc.* **1985**, *107*, 6410–6411.
(4) (a) Gassman, P. G.; Proehl, G. S. *J. Am. Chem. Soc.* **1980**, *102*, 6862–6863. (b) Mlinarić-Majerski, K.; Majerski, Z. *J. Am. Chem. Soc.* **1980**, *102*, 1418–1419. (c) Mlinarić-Majerski, K.; Majerski, Z. *J. Am. Chem. Soc.* **1983**, *105*, 7389–7395. (d) Vinković, V.; Majerski, Z. *J. Am. Chem. Soc.* **1982**, *104*, 4027–4029. (e) Morf, J.; Szeimies, G. *Tetrahedron Lett.* **1986**, *27*, 5363–5366. (f) Szeimies-Seebach, U.; Schöffer, A.; Römer, R.; Szeimies, G. *Chem. Ber.* **1981**, *114*, 1767–1785.
(5) (a) Wiberg, K. B.; Burgmaier, G. J. *Tetrahedron Lett.* **1969**, 317–319. (b) Gassman, P. G.; Topp, A.; Keller, J. W. *Tetrahedron Lett.* **1969**, 1093–1095. (c) Wiberg, K. B.; Burgmaier, G. J. *J. Am. Chem. Soc.* **1972**, *94*, 7396–7401. (d) Wiberg, K. B.; Pratt, W. E.; Bailey, W. F. *J. Am. Chem. Soc.* **1977**, *99*, 2297–2302.
(6) (a) Hamon, D. P. G.; Trenerry, V. C. *J. Am. Chem. Soc.* **1981**, *103*, 4962–4965. (b) Szeimies-Seebach, U.; Szeimies, G. *J. Am. Chem. Soc.* **1978**, *100*, 3966–3967. (c) Majerski, Z.; Žuanić, M. *J. Am. Chem. Soc.* **1987**, *109*, 3496–3498.
(7) Wiberg, K. B.; Walker, F. H.; Pratt, W. E.; Michl, J. *J. Am. Chem. Soc.* **1983**, *105*, 3638–3641. Reference 4e.
(8) (a) Walker, F. H.; Wiberg, K. B.; Michl, J. *J. Am. Chem. Soc.* **1982**, *104*, 2056–2057. (b) Carroll, W. F., Jr.; Peters, D. G. *J. Am. Chem. Soc.* **1980**, *102*, 4127–4134. Carroll, W. F., Jr.; Peters, D. G. *Tetrahedron Lett.* **1978**, 3543–3546. (c) Wiberg, K. B.; Bailey, W. F.; Jason, M. E. *J. Org. Chem.* **1976**, *41*, 2711–2714. References 5c,d.
(9) Eaton, P. E.; Temme, G. H., III *J. Am. Chem. Soc.* **1973**, *95*, 7508–7510.

0022-3263/88/1953-2662$01.50/0 © 1988 American Chemical Society