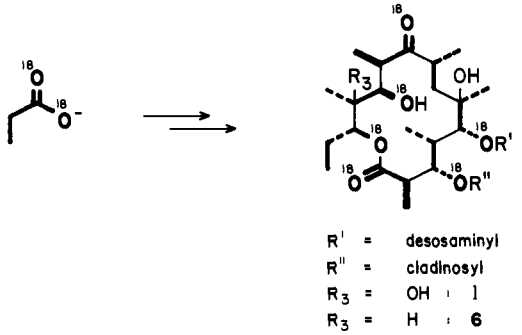
Scheme II



water was administered in portions (2 mL after 48-h, 3 mL after 72-h, and 5 mL after 96-h incubation time) to 100 mL of a growing fermentation of *Streptomyces erythreus*[19] in a complex cotton seed medium. After a total of 6 days, the resulting mixture of antibiotics was extracted with chloroform and the mixture of erythromycins A and B isolated as the readily crystallized 2'-benzoate esters. Separation of the two esters was achieved by chromatography on Sephadex LH 20 (1:1 chloroform-hexane)[11] to give 27 mg of erythromycin A 2'-benzoate and 16 mg of erythromycin B 2'-benzoate as well as 11 mg of a mixture of the two esters. Analysis of the 62.9-MHz ^{13}C NMR spectrum of each of the labeled macrolide esters established that carbons 1, 3, 5, 7, 9, 11, and 13 were labeled as expected.[7,20] The observed signal enhancements (ca. 13% ^{13}C per labeled site) were in accord with the enrichment calculated from the measured specific activity (2.59×10^6 dpm/mmol). A starter effect[6] was apparent in the slightly greater enhancement of the signals corresponding to C-13.[21]

Suitable conditions for incorporation of labeled propionate having been established, 100 mL of *S. erythreus* were fed a mixture of sodium [1-$^{18}O_2$,1-^{13}C]propionate[23] (0.333 g; 54.9% $^{18}O_2{}^{13}C$, 32.1% $^{18}O^{13}C$, 3.6% $^{16}O^{13}C$), 0.667 g unlabeled sodium propionate, and a trace of sodium [1-^{14}C]propionate and the resulting labeled erythromycins A and B were isolated and purified as the derived 2'-benzoates in the manner described above. The sites of ^{18}O enrichment were determined directly by ^{13}C NMR spectroscopy by taking advantage of the isotope shifts on the resonances of the attached ^{13}C nuclei, a technique recently introduced independently by Van Etten[24] and Vederas.[25] As summarized in Table I and illustrated in part in Figure 2, the peaks corresponding to C-1, -3, -5, -9, -11, and -13 in both erythromycin A and B benzoate each appeared as enhanced pairs of signals corresponding to ^{13}C-^{16}O and ^{13}C-^{18}O species, the latter resonances being shifted 0.02-0.05 ppm upfield, according to the type of C-O bond. From these results it is clear that each oxygen atom present in the first-formed macrolide aglycon, 6-deoxyerythronolide B (**3**), must have been derived largely from the precursor propionate (Scheme II). In particular, the four secondary hydroxyl sites (including C-13) all bore excess oxygen isotope, irrespective of their individual configurations. Interestingly although the carbonyl group at C-9 appeared to have undergone considerable oxygen exchange, a small amount of ^{18}O (ca 15%) was still evident. Variable but much smaller amounts of exchange had occurred at each of the remaining sites.

Since the tertiary hydroxyl groups at C-6 and C-12 of erythromycin A and at C-6 of erythromycin B have already been shown to originate from molecular oxygen,[10,11] the origin of all the macrolide aglycon oxygens has now been established. The above results clearly exclude both the oxidation pathway A and the dehydration-rehydration pathway C described earlier. Whether the stereochemical arrangement of the secondary methyl groups at C-2, -4, -8, -10, and -12 is determined by the choice of 2(*R*) or 2(*S*)-methylmalonyl-CoA as condensation substrate or whether condensation of a single enantiomer of methylmalonyl-CoA is followed in some instances by epimerization of the α-methyl-β-ketoacyl-CoA intermediate remains to be established. Nonetheless the stereochemical homology among the various known macrolides embodied by the Celmer Model emphasizes the potential generality of our results which are also completely in accord with related findings on the biosynthesis of the polyethers monensin[26] and lasalocid[27] described in the accompanying papers.

Acknowledgment. This work was supported by NIH Grant GM 22172. Strains of *S. erythreus* were kindly provided by Dr. L. E. Day of the Eli Lilly Co. who also furnished helpful information on fermentation conditions. A sample of erythromycin A was a generous gift from Eli Lilly. The [^{13}C]cyanide was obtained from the National Stable Isotopes Resource, Los Alamos Scientific Laboratory, supported by the U.S. DOE and the NIH (RR-00962). The Bruker WM 250 NMR spectrometer used in this work was purchased with funds provided by the NSF and the Montedison Group of Milan. We would like to thank Professor Richard Hutchinson of the University of Wisconsin for frequent mutual exchanges of information and for generously offering to delay publication of his own work[27] in order to allow simultaneous submission of our manuscripts.

(19) *S. erythreus*, Eli Lilly strain E57-236.

(20) The ^{13}C NMR spectra of erythromycins A and B have been completely assigned: J. G. Nourse and J. D. Roberts, *J. Am. Chem. Soc.*, **97**, 4584 (1975); Y. Terui, K. Tori, K. Nagashima, and N. Tsuji, *Tetrahedron Lett.*, 2583 (1975); S. Omura, A. Neszmelyi, M. Sangare, and G. Lukacs, *ibid.*, 2939 (1975).

(21) In agreement with these observations, Kuhn-Roth oxidation[22] of a sample of labeled erythromycin benzoate obtained from a separate feeding of 1.0 g of [1-^{14}C]propionate gave propionic acid whose *p*-phenylphenacyl ester bore 21.3% of the specific activity of the intact macrolide. The lack of activity in acetic acid isolated from the same Kuhn-Roth degradation established that no significant randomization of label had occurred.

(22) E. Wiesenberger, *Mikrochim. Acta*, **33**, 51 (1948).

(23) [1-^{13}C]Propionitrile was obtained by reacting 25.0 mmol of potassium [^{13}C]cyanide (90 atom%) with 25.1 mmol of ethyl iodide in 5.0 g of absolute methanol to which 0.24 mL of [^{18}O]water (95 atom %) had been added. (70 °C/12 h and then 80 °C/38 h). The entire mixture was distilled, mixed with 53 mmol of [^{18}O]water (95 atom %) and 29.5 mL of 0.85 M potassium *tert*-butoxide in *tert*-butyl alcohol, and refluxed for 48 h. The residue obtained upon evaporation of the solvent was redissolved in distilled water, acidified with phosphoric acid, and lyophilized. Titration of the lyophilizate with sodium hydroxide gave sodium [1-$^{18}O_2$,1-^{13}C]propionate (75% yield), a portion of which was converted to the *p*-phenylphenacyl ester for mass spectrometric analysis.

(24) J. M. Risley and R. L. Van Etten, *J. Am. Chem. Soc.*, **101**, 252 (1979); *ibid.*, **102**, 4609, 6699 (1980).

(25) J. C. Vederas, *J. Am. Chem. Soc.*, **102**, 374 (1980); J. C. Vederas and T. T. Nakashima, *J. Chem. Soc., Chem. Commun*, 183 (1980).

(26) D. E. Cane, T. C. Liang, and H. Hasler, *J. Am. Chem. Soc.*, following paper in this issue.

(27) C. R. Hutchinson, M. R. Sherman, J. C. Vederas, and T. T. Nakashima, *J. Am. Chem. Soc.*, first paper in this series; C. R. Huchinson, M. M. Sherman, J. C. Vederas, A. G. McInnes, and J. A. Walters, *ibid.*, preceding paper in this issue.

Polyether Biosynthesis. Origin of the Oxygen Atoms of Monensin A

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The polyether antibiotic monensin A (**1**), an important agent in the control of coccidiosis in poultry,[1] is one of a large class of naturally occurring ionophores which have attracted increasing

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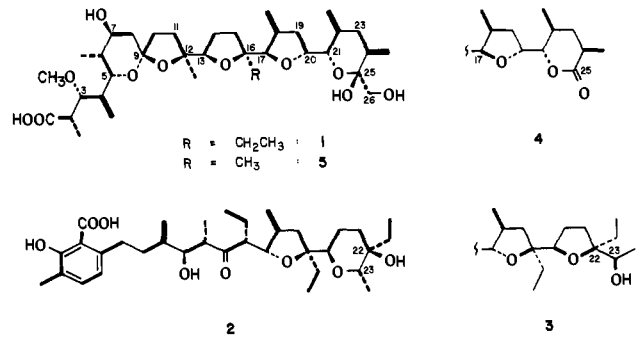
attention for their unusual chemical and biochemical properties.[2] Although the first such substance, nigericin, was isolated 30 years ago,[3] it was not until 1967 that the first structure, that of monensin A, was assigned.[4]

The gross features of the biosynthesis of the polyether antibiotics have been established over the last several years. These substances, often thought of as branched-chain polyhydroxy fatty acid derivatives, bear an obvious resemblance to the macrolides. The similarity is borne out by their apparent biogenesis which is based on the assembly of varying proportions of acetate, propionate, and butyrate precursors. Monensin (**1**) was in fact one of the first such substances to be subjected to biosynthetic investigations.[5] Experiments involving ^{14}C-labeled precursors followed by partial degradation of the derived monensin indicated that the carbon chain is assembled from five acetate, seven propionate, and one butyrate molecule. (Scheme I). Attempts to incorporate [^{13}C]acetate failed to give a sufficient enrichment for detection by ^{13}C NMR spectroscopy. Experiments with lasalocid A (**2**), initially based on incorporation of ^{14}C precursors and later using ^{13}C NMR techniques, confirmed the expected origin from five acetates, four propionates,, and three butyrates.[6] On the basis of isolation of a minor cometabolite, isolasalocid A (**3**), which differs from lasalocid A in the size of the terminal ring and the stereochemistry at C-22 and C-23, Westley has made the intriguing suggestion that the various tetrahydrofuran and tetrahydropyran rings of the polyethers are formed by sequential opening of a polyepoxide precursor.[7] It was also found that lasalocid A is accompanied by small amounts of the homologues lasalocids B–E in which one of the four propionates has been replaced by a butyrate precursor.[8] More recently ^{13}C NMR biosynthetic studies have been reported for the polyethers narisin,[9] salinomycin,[10] lysocellin,[11] and lonomycin A.[12]

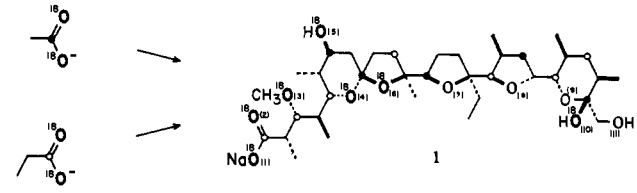
Because of the similar biogenesis of macrolides and polyethers we wished to determine the biosynthetic origins of the attached oxygen atoms of monensin A in order to begin to understand the details of how the intricate polyether skeleton is assembled. Using high-resolution ^{13}C NMR spectroscopy, we have now directly confirmed the acetate and propionate origins of monensin and identified the source of 7 of the 11 skeletal oxygen atoms, as described below.

Although ^{13}C NMR assignments have been reported for several polyethers and a set of empirical assignment rules has been proposed,[13] the ^{13}C NMR spectrum of monensin A itself has yet to be assigned. We therefore assigned the oxygen-bearing carbons

Scheme I



Scheme II



of monensin A sodium in the following manner. The quaternary carboxylate (C-1, 181.1 ppm), ketal (C-9, 107.0), and hemiketal (C-25, 98.2) carbon signals were readily identified on the basis of their characteristic chemical shifts as well as the absence of the 98.2-ppm signal in the spectrum of the periodate oxidation product **4**.[4] The remaining two quaternary carbon signals corresponding to C-12 and C-16 appeared at 85.2 and 85.8 ppm and were not separately assigned. The signal at 64.8 ppm which appeared as a triplet in the off-resonance decoupled spectrum corresponded to C-26 while the C-7 carbinyl carbon (70.1 ppm) was readily identified by its characteristic 2.8-ppm downfield shift upon acetylation. The six remaining carbinyl carbons were assigned by a series of single-frequency off-resonance decoupling experiments and analysis by the method of Birdsall.[14] The corresponding critical proton assignments were made with the aid of proton–proton decoupling of the 270-MHz ^1H NMR spectrum. For example C-3 (83.2 ppm) was correlated with H-3 which appeared as a double doublet ($J_{3,2}$ = 10.3, $J_{3,4}$ = 2.0 Hz) at δ 3.19, coupled to H-2 (δ 2.53) and H-4. Similarly C-17 (84.8 ppm) was identified by correlation with H-17, a doublet (J = 3.5 Hz) at δ 3.94. The corresponding signal for C-17 in monensin B (**5**), which bears a methyl rather than an ethyl group at the adjacent C-16,[15] was shifted downfield to 86.5 ppm. In like manner the signals corresponding to C-5 (68.2), C-13 (82.8), C-20 (76.3), and C-21 (74.4) were unambiguously assigned as was the methoxyl carbon at 57.7 ppm.

Following preliminary incorporations of ^{14}C-labeled substrates, cultures of *Streptomyces cinnamonensis*[16,17] were fed samples of sodium [1-^{13}C]acetate and [1-^{13}C]propionate in separate experiments according to a protocol similar to that described below for ^{18}O-labeled precursors. Analysis of the ^{13}C NMR spectra of the resulting labeled monensins revealed that the signals corresponding to C-7, C-9, C-13, C-25, and (presumably) C-19 had been significantly (3%) enhanced in the monensin A sample derived from [1-^{13}C]acetate, whereas 4% enrichments at C-1, C-3, C-5, C-17, C-21, and (presumably) C-11 and C-23 resulted from incorporation of [1-^{13}C]propionate. These results provided direct evidence

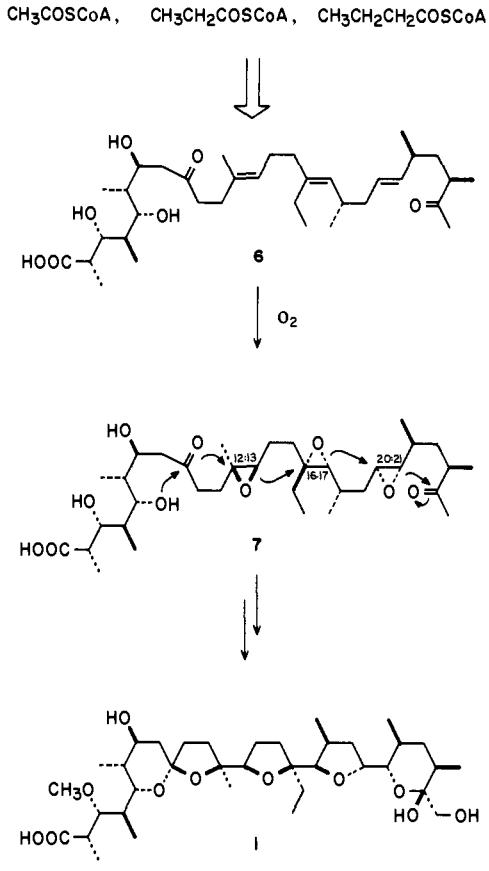
(1) R. F. Shumard and M. E. Callender, *Antimicrob. Agents Chemother.*, 369 (1968).
(2) Reviews: J. W. Westley, *Annu. Rep. Med. Chem.*, **10**, 246 (1975); J. W. Westley, *Adv. Appl. Microbiol.*, **22**, 177 (1977).
(3) R. L. Harned, P. Harten, C. J. Corum, and K. L. Jones, *Antibiot. Chemother. (Washington, D.C.)*, **1**, 594 (1951); J. Berger, A. I. Rachlin, W. E. Scott, L. H. Sternbach, and M. W. Goldberg, *J. Am. Chem. Soc.*, **73**, 5295 (1951).
(4) A. Agtarap, J. W. Chamberlin, M. Pinkerton, and L. Steinrauf, *J. Am. Chem. Soc.*, **89**, 5737 (1967); M. E. Haney and M. M. Hoehn, *Antimicrob. Agents Chemother.*, 349 (1968); A. Agtarap and J. W. Chamberlin, *ibid.*, 359 (1968); W. K. Lutz, F. K. Winkler, and J. D. Dunitz, *Helv. Chim. Acta*, **54**, 1103 (1971); M. Pinkerton and L. K. Steinrauf, *J. Mol. Biol.*, **49**, 533 (1970).
(5) L. E. Day, J. W. Chamberlin, E. Z. Gordee, S. Chen, M. Gorman, R. L. Hamill, T. Ness, R. E. Weeks, and R. Stroshane, *Antimicrob. Agents Chemother.*, 410 (1973).
(6) J. W. Westley, R. H. Evans, G. Harvey, R. G. Pitcher, D. L. Pruess, A. Stempel, and J. Berger, *J. Antibiot.*, **27**, 288 (1974); H. Seto, J. W. Westley, and R. G. Pitcher, *ibid.*, **31**, 288 (1978); J. W. Westley, D. L. Pruess, and R. G. Pitcher, *J. Chem. Soc., Chem. Commun.*, 161 (1972).
(7) J. W. Westley, J. F. Blount, R. H. Evans, A. Stempel, and J. Berger, *J. Antibiot.*, **27**, 597 (1974).
(8) J. W. Westley, W. Benz, J. Donahue, R. H. Evans, C. G. Scott, A. Stempel, and J. Berger, *J. Antibiot.*, **27**, 744 (1974).
(9) D. E. Dorman, J. W. Paschal, W. M. Nakatsukasa, L. L. Huckstep, and N. Neuss, *Helv. Chim. Acta*, **59**, 2625 (1976).
(10) H. Seto, Y. Miyazaki, K. Fujita, and N. Otake, *Tetrahedron Lett.*, 2417 (1977).
(11) N. Otake, H. Seto, and M. Koenuma, *Agric. Biol. Chem.*, **42**, 1879 (1978).
(12) H. Seto, K. Mizoue, N. Otake, M. Yamagishi, T. Mizutani, H. Hara, and S. Omura, *J. Antibiot.*, **31**, 931 (1978).
(13) H. Seto, K. Mizoue, H. Nakayama, K. Furihata, N. Otake, and H. Yonehara, *J. Antibiot.*, **32**, 239 (1979); H. Seto, H. Nakayama, T. Ogita, K. Furihata, K. Mizoue, and N. Otake, *ibid.*, **32**, 244 (1979).
(14) B. Birdsall, N. J. M. Birdsall, and J. Feeney, *Chem. Commun.*, 316 (1972).
(15) M. Gorman, J. W. Chamberlin, and R. L. Hamill, *Antimicrob. Agents Chemother.*, 363 (1968).
(16) *S. cinnamonensis*, Eli Lilly strain A3823.5.
(17) W. M. Stark, N. G. Knox, and J. E. Westhead, *Antimicrob. Agents Chemother.*, 353 (1968).

Table I. Incorporation of [1-$^{18}O_2$,1-^{13}C]Acetate and [1-$^{18}O_2$,1-^{13}C]Propionate into Monensin A

	precursor						
	[1-$^{18}O_2$,1-^{13}C]acetate			[1-$^{18}O_2$,1-^{13}C]propionate			
C	^{13}C shift, ppm[a,b,e]	$\Delta\delta$, ppm[g]	$^{18}O/^{16}O$[h]	C	^{13}C shift, ppm[a,b,e]	$\Delta\delta$, ppm[g]	$^{18}O/^{16}O$[h]
7	70.46	0.02	60:40	1	181.05	0.03	20:80
9	106.98	0.03	60:40	3	83.06	0.03	40:60
13	82.48	0.0	0:100	5	68.30	0.03	40:60
19	33.30			11	33.22		
25	98.25	0.02	55:45	17	84.90	0.0	0:100
				21	74.45	0.0	0:100
				23	35.71		

[a] Bruker WM 250, 62.9 MHz; spectral width 12 000 Hz; 64K data points; quadrature detection; 55° pulse; 5200 transients; 0.049 g in 2.0 mL of $CDCl_3$. Resolution enhancement was achieved by Lorentz-Gauss multiplication of FID prior to Fourier transformation [R. R. Ernst, *Adv. Magn. Reson.*, **2**, 59 (1966)], -1.0-Hz line broadening, 0.4 Gaussian multipler; 0.006 ppm/data point. [b] Monensin A sodium, 8.7×10^5 dpm/mmol. [c] 62.9 MHz; spectral width 12 000 Hz, 64K data points, quadrature detection, 55° pulse, 12 550 transients; 0.022 g in 2.0 mL of $CDCl_3$; -1.0-Hz line broadening, 0.3 Gaussian multiplier; 0.006 ppm/data point. [d] Monensin A sodium, 4.5×10^6 dpm/mmol. [e] Average ^{13}C enrichment, 3.1 ± 1.1%. [f] Average ^{13}C enrichment, 4.0 ± 0.2%. [g] $^{13}C^{18}O$ isotope shift, ±0.005 ppm. [h] Uncorrected for contribution of natural abundance ^{13}C to $^{13}C^{16}O$ peak; ±5.

Scheme III



for the precursor–product relationship inferred from the original radioisotope incorporation and degradation studies.[5]

With the above results in hand, a mixture of 0.200 g of sodium [1-$^{18}O_2$,1-^{13}C]propionate[18] (54.9% $^{18}O_2{}^{13}C$, 32.1% $^{18}O^{13}C$, 3.6% $^{16}O^{13}C$),[19] 0.800 g of unlabeled sodium propionate,[20] and 2.2×10^7 dpm of sodium [1-^{14}C]propionate as internal standard dissolved in 10.0 mL of water was dispensed in lots of 2.0, 1.5, and 1.5 mL to each of two 50-mL fermentation cultures[17] of *S. cinnamonensis* after periods of 48-, 72-, and 96-h incubation, respectively, at 32 °C. Fermentation was continued for an additional 3 days after which the resulting crude monensin sodium was extracted into chloroform according to the literature procedure[5,17] and purified by successive silica gel column and thin-layer chromatography to give 0.022 g of monensin A, 4.5×10^6 dpm/mmol. In the corresponding high-resolution 62.9-MHz ^{13}C NMR spectrum, the peaks derived from C-1, C-3, and C-5 each appeared as an enhanced pair of signals corresponding to the respective ^{13}C–^{16}O and ^{13}C–^{18}O species[21,22] (Scheme II). As summarized in Table I the observed ^{18}O enrichments deviated slightly from the maximum theoretical enrichment of 78%, reflecting varying degrees of oxygen exchange at each site, the greatest amount having occurred at C-1. Interestingly no ^{18}O was present at either C-17 or C-21 whose ^{13}C NMR signals each appeared as enhanced singlets as did those for the nonoxygen-bearing carbons, C-11 and C-23. The significance of these results is discussed below. Using the same feeding regimen, a mixture of 0.200 g of sodium [1-$^{18}O_2$,1-^{13}C]acetate[23] (73.4% $^{18}O_2{}^{13}C$, 14.5% $^{18}O^{13}C$, 0.8% $^{16}O^{13}C$),[19] 0.800 g of unlabeled sodium acetate,[20] and 7.34×10^6 dpm of sodium [2-^{14}C]acetate was administered to *S. cinnamonensis* and the derived labeled monensin A isolated and purified in the usual manner. Analysis of the 62.9-MHz ^{13}C NMR spectrum revealed the presence of excess ^{18}O at C-7, C-9, and C-25, as evidenced by the usual pair of signals, whereas no ^{18}O attached to C-13 was apparent. The signals corresponding to C-13 and C-19 appeared as enhanced but unsplit singlets. Since the tetrahydropyranyl oxygen [O(4)][24] of the C-9 spiroketal had been shown to be derived from the C-5 propionate unit, the acetate-derived oxygen at C-9 was assigned to the tetrahydrofuran moiety [O(6)]. The ^{18}O attached to C-25 was assigned to the hemiketal hydroxyl oxygen [O(10)] by analysis of the lactone **4** obtained by sodium periodate cleavage of the vicinal diol.[4] The signal corresponding to C-25 of **4** appeared as a pair of peaks separated by 0.04 ppm, characteristic of ^{18}O in the carbonyl as distinguished from the ether oxygen.[12,22]

The above results demonstrate that 7 of the 11 oxygen atoms of monensin A are derived directly from the carboxylate oxygens of the precursors acetate and propionate. As we have found for the macrolide erythromycin A,[18] the source of the oxygen atoms is not correlated with the eventual D (C-3) or L (C-5 and C-7) configuration[25] of the individual hydroxyl functions, indicating

(18) D. E. Cane, H. Hasler, and T. C. Liang, *J. Am. Chem. Soc.*, preceding paper in this issue.

(19) Enrichments in $^{18}O^{13}C$ were determined by mass spectrometry on the derived *p*-phenylphenacyl ester.

(20) The ^{13}C-labeled precursor was diluted with unlabeled substrate in order to avoid excess intramolecular multiple labeling of the polyether product which results in undesirable broadening of the resultant ^{13}C NMR resonances due to ^2J(C–C) couplings, thereby obscuring the 0.02–0.04-ppm isotopic shifts.

(21) J. M. Risley and R. L. Van Etten, *J. Am. Chem. Soc.*, **101**, 252 (1979); *ibid.*, **102**, 4609, 6699 (1980).

(22) J. C. Vederas, *J. Am. Chem. Soc.*, **102**, 374 (1980); J. C. Vederas and T. T. Nakashima, *J. Chem. Soc., Chem. Commun.*, 183 (1980).

(23) Sodium [1-$^{18}O_2$,1-^{13}C]acetate was prepared in 88% overall yield starting with 16 mmol of methyl iodide and 16 mmol of potassium [^{13}C]-cyanide (90 atom %) by a procedure similar to that used for the preparation of the corresponding labeled propionate (footnote 23, ref 18).

(24) The oxygen numbering system is that suggested by Westley.[2]

(25) Fisher–Klyne notation: W. Klyne, *Chem. Ind. (London)*, 1022 (1951); E. Eliel, "Stereochemistry of Carbon Compounds", McGraw-Hill, New York, 1962, pp 91–92.

that in the biosynthesis of the growing polyhydroxy fatty acid chain, β-ketoacyl CoA reduction can occur with either stereochemistry. For the moment no information is available as to how the secondary methyl stereochemistry at C-2, C-4, C-6, and C-24 is set.[26] While further experiments, now in progress, will be required to determine directly the origins of the three remaining ether oxygens as well as that of the C-26 hydroxyl, it is likely that all four atoms are derived from molecular oxygen. Extending the original suggestion of Westley,[7] it is therefore interesting to speculate that the first formed polyfunctional fatty acid would be the all-(*E*)-triene **6**[27] which could undergo epoxidation to give the (12*R*,13*R*,16*R*,17*R*,20*S*,21*S*)-triepoxide **7** (Scheme III). Attack of the C-5 hydroxyl of **7** at the C-9 carbonyl carbon would initiate a cascade of ring closures to generate all five ether rings of monensin. These results are also in accord with similar findings by Hutchinson et al. on the biosynthesis of the aromatic polyether lasalocid, reported in the accompanying communications.[29]

Acknowledgment. This work was supported by NIH Grant GM 22172. Strains of *S. cinnamonensis* as well as samples of monensin were generous gifts of the Eli Lilly Co. The [^{13}C]cyanide was obtained from the National Stable Isotopes Resource, Los Alamos Scientific Laboratory, supported by the U.S. DOE and the NIH (RR-00962). The Bruker WM 250 NMR spectrometer used in this study was purchased with funds provided by the NSF and the Montedison Group of Milan. We would like to thank Professor Richard Hutchinson of the University of Wisconsin for frequent mutual exchanges of information and for generously offering to delay publication of his own work[29] in order to allow simultaneous submission of our manuscripts.

(26) The methyl group stereochemistry at C-18 and C-22 is presumably determined as a consequence of the reduction of α-methyl, α,β-unsaturated-acyl-CoA intermediates, analogous to those of normal fatty acid biosynthesis: F. Lynen, *Biochem. J.*, **102**, 381 (1967); K. Bloch and D. Vance, *Annu. Rev. Biochem.*, **46**, 263 (1977); B. Sedgwick and J. W. Cornforth, *Eur. J. Biochem.*, **75**, 465 (1977); B. Sedgwick, J. W. Cornforth, S. J. French, R. T. Gray, E. Kelstrup, and P. Willadsen, *ibid.*, **75**, 481 (1977); B. Sedgwick, C. Morris, and S. J. French, *J. Chem. Soc., Chem. Commun.*, 193 (1978).

(27) Each (*E*)-olefin could be formed by either syn dehydration of an *erythro*-α-alkyl-β-hydroxyacyl-CoA or anti dehydration of the corresponding *threo*-α-alkyl-β-hydroxyacyl-CoA. In the latter regard we note the threo relationship of the C-2,-3, C-4,-5, and C-6,-7 centers of monensin itself. The same threo stereochemistry is prevalent in all known macrolides, as summarized by Celmer's rules.[28]

(28) W. D. Celmer, *Pure Appl. Chem.*, **28**, 413 (1971); W. D. Celmer, *J. Am. Chem. Soc.*, **87**, 1801 (1965).

(29) C. R. Hutchinson, M. M. Sherman, J. C. Vederas, and T. T. Nakashima, *J. Am. Chem. Soc.*, first paper in this series; C. R. Hutchinson, M. M. Sherman, J. C. Vederas, A. G. McInnes, and J. A. Walters, *ibid.*, second paper in this series.

Reaction of Alkoxides with (Phenylthio)carbene: A Novel Oxyanionic Substituent Effect on the C–H Insertion of Carbenes

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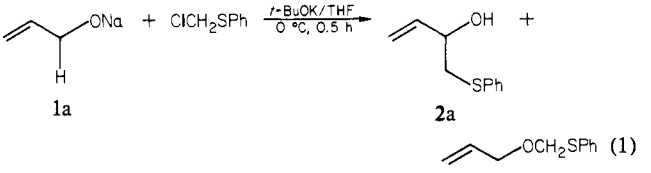
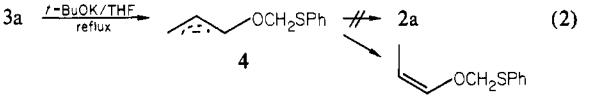
While the addition of carbenes to alkenes has been utilized as an efficient tool for C–C bond formation in organic synthesis, the insertion of carbenes has been overlooked owing to its less selective nature.[1] An activation of a particular C–H bond seems to be a requisite for the selective insertion to occur. For example, Seyferth et al. have reported that the α-C–H bond of tetrahydrofuran shows enhanced reactivity toward dichlorocarbene generated from $PhHgCCl_2Br$ to give the insertion product in high yield.[2] In connection with the current interest in oxyanionic substituent effects,[3] we have examined the reactivity of carbenes toward alkoxide anions. Herein we wish to report a novel effect of oxyanion substituent which greatly facilitates the insertion of (phenylthio)carbene[4] into the α-C–H bond of alkoxide anions.[5]

To a mixed suspension of sodium allylalkoxide (**1a**, 2.5 equiv) and *t*-BuOK (2.5 equiv) in THF was added a THF solution of chloromethyl phenyl sulfide, and the total mixture was stirred for 0.5 h at 0 °C. After aqueous workup followed by column chromatography, 1-(phenylthio)-3-buten-2-ol (**2a**) and allyl (phenylthio)methyl ether (**3a**) were isolated in 29% and 64% yield, respectively (eq 1). The formal insertion product **2a** could be



1a **2a** **3a** (1)

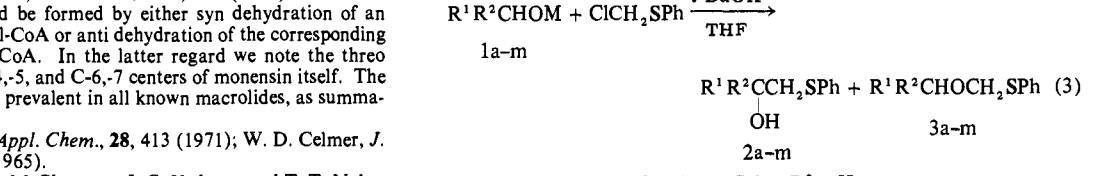
produced via a Wittig rearrangement of the first formed ether **3a** as shown in eq 2. However, the possibility is ruled out as



(2)

follows: **3a** slowly isomerized to *cis*-1-propenyl (phenylthio)methyl ether without the formation of **2a** when treated with *t*-BuOK (1.25 equiv) in refluxing THF.[8] Thus, under these reaction conditions allylic carbanion **4**, if formed, cannot be rearranged to give **2a**.

Not only other allylic alkoxides but also propargylic, benzylic, and even simple alkyl alkoxides undergo C–H insertion reaction to give β-phenylthio alcohols **2a–m** besides forming the corresponding ether **3a–m** (eq 3). These results are summarized in Table I.



(3)

a, $R^1 = CH_2{=}CH-$; $R^2 = H$
b, R^1 = *trans*-$CH_3CH{=}CH-$; $R^2 = H$
c, R^1 = *trans*-$PhCH{=}CH-$; $R^2 = H$
d, $R^1 = CH_2{=}C(CH_3)-$; $R^2 = H$
e, $R^1 = (CH_3)_2C{=}CH-$; $R^2 = H$
f, $R^1 = CH_2{=}CH-$; $R^1 = CH_3$
g, $R^1 = HC{\equiv}C-$; $R^2 = H$
h, $R^1 = Ph-$; $R^2 = H$
i, $R^1 = Et$; $R^2 = H$
j, $R^1 = CH_3$; $R^2 = CH_3$

While a small amount of olefin addition product **5** (13%) was obtained in addition to the insertion product **2e** in the reaction

(1) (a) Kirmse, W. "Carbene Chemistry", 2nd ed.; Academic Press: New York, 1971. (b) Parham, E. P.; Schweizer, E. E. *Org. React.* **1963**, *13*, 55. (c) Burke, S. D.; Grieco, P. A. *Ibid.* **1971**, *26*, 361. (d) Simmons, H. E.; Cairns, T. L.; Vladuchick, S. A.; Hoiness, C. M. *Ibid.* **1973**, *20*, 1.

(2) Seyferth, D.; Mai, V. A.; Gordon, M. E. *J. Org. Chem.* **1970**, *35*, 1993.

(3) (a) Evans, D. A.; Baillargeon, D. J. *Tetrahedron Lett.* **1978**, 3315 and 3319, and references cited therein. (b) Steigerwald, M. L.; Goddard, W. A., III; Evans, D. A. *J. Am. Chem. Soc.* **1979**, *101*, 1994.

(4) (a) For organosulfur carbenes and carbenoids see, for example: Block, E. "Reaction of Organosulfur Compounds"; Academic Press: New York, 1978. (b) Schöllkopf, U.; Lehmann, G. J.; Paust, J.; Härtl, H.-D. *Chem. Ber.* **1964**, *97*, 1527. (c) Boche, G.; Schneider, D. R. *Tetrahedron Lett.* **1975**, 4247.

(5) Under the reaction conditions of the Williamson synthesis, observations of complex reactions have sometimes been reported. For example, Corey et al. have noted a complex reaction between iodomethyl methylsulfide and secondary or tertiary sodium alkoxides.[6] Carig et al. have detected the C–H insertion product of vinylidenecarbene when they treated 1-bromoalk-1-ynes or 1-bromo 1,2-dienes with sodium alkoxides.[7]

(6) Corey, E. J.; Bock, M. G. *Tetrahedron Lett.* **1975**, 3269.

(7) (a) Craig, J. C.; Beard, C. D. *Chem. Commun.* **1971**, 693. (d) *J. Am. Chem. Soc.* **1974**, *96*, 7950.

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