Kylinone, a New Sesquiterpene Skeleton from the Marine Alga *Laurencia pacifica*

Scott J. Selover and Phillip Crews*

Thimann Laboratories, University of California, Santa Cruz, California 95064

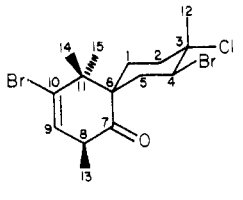
Received May 7, 1979

The structure of a new spirobicycloundecane sesquiterpene, kylinone (1), is reported. The relative configurations at all four chiral centers have been assigned as 3*S**, 4*S**, 6*R**, and 8*S** on the basis of a combination of spectroscopic and chemical observations. Kylinone (1) has been synthesized from a known chamigrene, deoxyprepacifenol (7), by epoxide opening accompanied by a methyl migration. Epoxide openings in the other related spirobicyclic undecanes 8 and 10 are also discussed.

The red seaweed genus *Laurencia* is, at present, unsurpassed as a source of halogenated sesquiterpenes.[1] Some time ago we began to explore the comparative chemistry of *Laurencia pacifica* (Kylin) and its associated epiphyte *Erythrocystis saccata* (J. Agardh). Not surprisingly, we were able to observe several known halogenated sesquiterpenes as major components from the extracts of *L. pacifica*. Many of these same natural products were also components of extracts from *E. saccata* which had been carefully detached from its host *L. pacifica*.[2] Among the minor components from the *Laurencia* extracts was a unique sesquiterpene, kylinone (1). Its structure and some aspects of its chemistry are reported below.

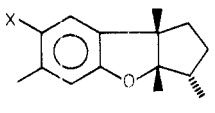
Results and Discussion

Methanol extraction of *L. pacifica* collected during February 1978 at Stillwater Cove on the Monterey Peninsula, CA, afforded kylinone (1) along with several known

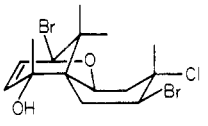


1

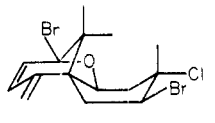
sesquiterpenes including aplysin (2),[3] debromoaplysin (3),[3] pacifenol (4),[4] and pacifidiene (5).[4,5] One other sesquiterpene, debromolaurinterol (6), was not completely purified but could be observed by NMR and GC/MS.[6]



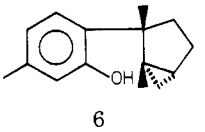
2, X = Br
3, X = H



4



5



6

Kylinone (1), IR 1725 cm^{-1}, displayed a mass spectral parent ion ($C_{15}H_{21}OBr_2Cl$; cluster at *m/e* 410, 412, 414, 416) and an exact mass of *m/e* 375.9868 ($M^+ - HCl$; calcd for $C_{15}H_{20}O^{79}Br^{81}Br$, *m/e* 375.9861). The low-field region in the ^{13}C NMR showed a carbonyl signal at δ 209.9 and trisubstituted double bond signals at δ 130.0 (d) and 136.6 (s). This, along with the molecular formula, required that 1 be bicyclic. Two of the three halogens were assumed to be attached to aliphatic carbons on the basis of ^{13}C NMR lines at δ 70.8 (s) and 60.6 (d). Four methyls were also observable at δ 24.8 (q), 24.0 (q), 23.8 (q), and 14.2 (q). Both the gross structure of the two rings and the point of attachment of the methyls to these rings were deduced from several pieces of spectral data.

A monomethyl ring was assembled as shown in 1 on the basis of the similarity of NMR properties of 1 to naturally occurring chamigrene derivatives 7 and 8 from *Laurencia*.[11,17] This is best shown in Figure 1 by ^1H resonances for 1 at δ 1.67 (CH_3, s), 2.33 (H_{2e}, ddd, apparent J = 13, 4, 3 Hz), 4.66 (H_{4a}, d of d, apparent J = 12.2, 4.9 Hz) coupled to 2.67 (H_{5e}, ddd, apparent J = 13.6, 4.9, 2.5 Hz), and 1.80 (H_{5a}, t, apparent J = 12.2, 13.6 Hz). A common feature of the coupling to H_{5e} in 1, 7, and 8 was the long-range ^4J = 2.5 Hz from H_{1e}.

Important in the assignment of the trimethyl ring was the mass spectral base peak at *m/e* 174, 176 [1:1, $(C_7H_{11}Br)^+$]. This facile ion fragmentation could be explained by a retro-Diels–Alder cleavage to a $[CH_3CH{=}CHCBr{=}C(CH_3)_2]^+$ unit. A $(CH_3)_2C{-}CBr{=}CH{-}CH{-}CH_3$ piece within the cyclohexene precursor to this fragment could be easily seen from ^1H NMR resonances, including two CH_3 singlets at δ 0.90 and 1.20, a CH_3 doublet at δ 1.14 (J = 7.1 Hz) coupled to a one-H doublet of quartets at δ 3.05 (H_8, J = 7.1, 3.2 Hz) which was further coupled to a one-H doublet at δ 5.95 (H_9, J = 3.2 Hz) (irradiation at δ 3.05 collapsed the doublets at δ 5.95 (1 H) and δ 1.14 (3 H) to singlets). Attachment of the carbonyl to the methine carbon of the above subunit was favored, because the rather low-field shift of H_8 (δ 3.05) was only consistent with it being adjacent to both a double bond and a carbonyl.[7] Additional support for an unconjugated enone came from both the C=O IR frequency and the ^{13}C shift position of 1 compared to those of 3-bromo-2-cyclohexenone [IR 1675 cm^{-1}; ^{13}C NMR δ 194.7 (s)]. These features were all combined to define the trimethyl ring and

(1) J. D. Martin and J. Darias in "Marine Natural Products—Chemical and Biological Perspectives", Vol. 1, P. J. Scheuer, Ed., Academic Press, New York, 1978, Chapter 3.
(2) P. Crews, S. J. Selover, and L. J. Goff, unpublished results.
(3) (a) S. Yamamura and Y. Hirata, *Tetrahedron*, **19**, 1485 (1963); (b) T. Irie, M. Suzuki, and Y. Hayakawa, *Bull. Chem. Soc. Jpn.*, **42**, 843 (1969).
(4) J. J. Sims, W. Fenical, R. M. Wing, and P. Radlick, *J. Am. Chem. Soc.*, **95**, 972 (1973); **93**, 3774 (1971).
(5) M. O. Stallard and D. J. Faulkner, *Comp. Biochem. Physiol. B*, **49**, 25 (1974).
(6) T. Irie, M. Suzuki, E. Kurosawa, and T. Masamune, *Tetrahedron*, **26**, 3271 (1970).
(7) For example, compare the shifts of δ 3.0 ($C{=}CCH_2CO$) of (+)-10-hydroxy-4,10-dimethyl-(*E*)-4,11-dodecadien-2-one[8] vs. δ 2.05 ($C{=}CCH_2C$) of 2-methyl-1-pentene[9] and δ 2.3 (CCH_2CO) of 4-methyl-2-pentanone.[9]
(8) M. P. Cooke, Jr., *J. Org. Chem.*, **44**, 2461 (1979).
(9) C. J. Pouchert and J. R. Campbell, "The Aldrich Library of NMR Spectra", Vol. 2, Aldrich Chemical Co., Milwaukee, WI, 1974.

0022-3263/80/1945-0069$01.00/0

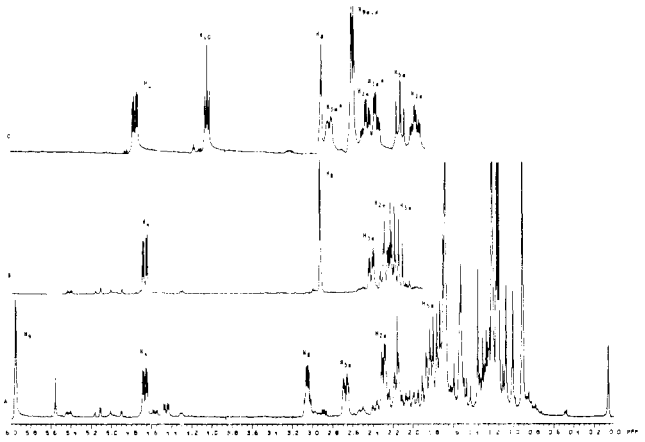
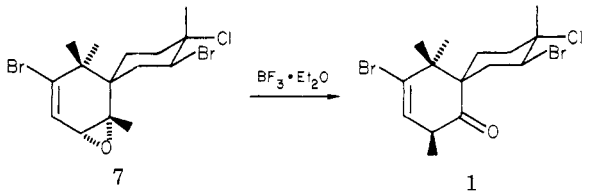


Figure 1. ^1H NMR (360 MHz, $CDCl_3$): A, kylinone 1; B, deoxyprepacifenol 7; C, chamigrene epoxide 8. The asterisk indicates that the assignment may be reversed.

the entire gross structure of kylinone as shown in **1**.

Turning to stereochemical considerations, the axial placement of the CH_3 at C_3 could be easily decided by applying our ^{13}C shift increment values.[10] The calculated shift of this axial methyl (ax = 26 ppm, eq = 32 ppm) was in good agreement with its observed value of δ 24.0. The equatorial C_4–Br was set by the ^1H J values discussed above. The stereochemistry at C_6 and C_8 relative to that at C_3 (or C_4), however, could not be solved on the basis of spectral arguments. These were resolved by converting deoxyprepacifenol (**7**), whose structure had been estab-

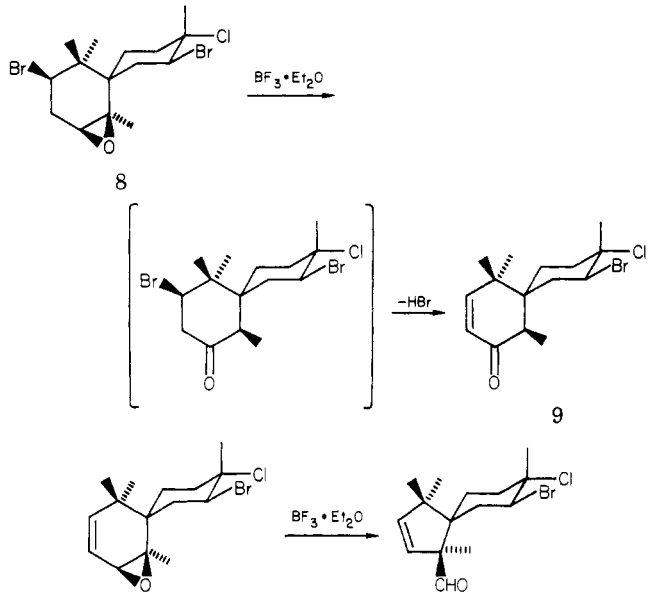


7 **1**

lished by X-ray crystallography,[11] to kylinone (**1**). Treatment of **7** with boron trifluoride etherate at room temperature for 18 h afforded **1** in high yield.

The literature contains numerous examples of acid-catalyzed epoxide openings in alicycles yielding carbonyl compounds by a stereospecific, suprafacial migration of a H,[14a-d] CH_3,[14e,f] alkyl,[14g] or ring carbon.[14h,i] Assuming the epoxide opening of **7** occurs via a haloallylic conjugated cation and a stereospecific CH_3 migration, the relative configuration of **1**[15] could be defined as 3S*, 4S*, 6R*, and 8S*.[16]

In connection with our study of the epoxide opening of **7** we examined some related cases. When epoxide **8** is



8 **9** **10** **11**

treated with boron trifluoride etherate, the epoxide opens, accompanied by H migration and dehydrohalogenation to the same enone **9** that Fenical and Howard observed from **8** with p-toluenesulfonic acid in benzene.[17] It should be noted that the proton at C_9 of this enone shows no coupling to the proton at C_7. Treatment of **10** under identical conditions yielded the ring-contracted product **11**. Comparable situations are the BF_3-catalyzed epoxide openings observed by Faulkner[18] for tridachione (**12**) which proceeded by H migration and by Hadley[14h] for 3-hydroxy-4,4,8,9,10-pentamethyldecalin 8,9-epoxide (**14**) which proceeded by ring contraction.

These results present some interesting contrasts about the course of epoxide openings for substituted 1-methylcyclohexene oxides. Comparing the products from **7**, **10**, and **12** reveals that a secondary allyl cation may need to be further activated by a halogen in order to dominate over a tertiary cation in influencing the direction of epoxide opening.[19] Further, once a cation intermediate is formed adjacent to a methyl as in intermediates from **7** and **14**,[20]

(10) P. Crews and E. Kho-Wiseman, *Tetrahedron Lett.*, 2483 (1978).

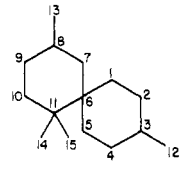
(11) C. Ireland, M. O. Stallard, D. J. Faulkner, J. Finer, and J. Clardy, *J. Org. Chem.*, **41**, 2461 (1976). The absolute stereochemistry reported here by the statistical method of comparing data for Friedal pairs may not be entirely secure in view of recent anomalous observations.[12,13]

(12) W. Fenical, B. Howard, K. B. Gifkins, and J. Clardy, *Tetrahedron Lett.*, 3983 (1975).

(13) Compare the enantiomeric-like absolute configurations of two sesquiterpenes from *Centaurea* by W. E. Thiessen and H. Hope [*Acta Crystallogr., Sect. B*, **26**, 554 (1970)] vs. those of J. Harley-Mason, A. T. Hewson, O. Kennard, and R. C. Peterson [*J. Chem. Soc., Chem. Commun.*, 460 (1972)].

(14) (a) B. N. Blackett, J. M. Coxon, M. P. Hartshorn, and K. E. Richards, *Tetrahedron*, **25**, 4999 (1969); (b) T. Petrzilka, K. P. Prasad, and G. Schmid, *Helv. Chim. Acta*, **59**, 1963 (1976); (c) J. E. McMurry, J. H. Musser, M. S. Ahmad, and L. C. Blaszczak, *J. Org. Chem.*, **40**, 1829 (1975); (d) K. M. Baker, L. H. Briggs, J. G. Buchanan, R. C. Cambie, B. R. Davis, R. C. Hayward, G. A. S. Long, and P. S. Rutledge, *J. Chem. Soc., Perkin Trans. 1*, 190 (1972); (e) J. Bascoul and A. C. de Paulit, *Bull. Soc. Chim. Fr.*, 189 (1969); (f) P. A. Bartlett and W. S. Johnson, *J. Am. Chem. Soc.*, **95**, 7501 (1973); (g) G. Berti, F. Bottari, A. Marsili, I. Morelli, and A. Mandelbaum, *Tetrahedron Lett.*, 529 (1968); (h) M. S. Hadley and T. G. Halsall, *J. Chem. Soc., Perkin Trans. 1*, 1334 (1974); (i) J. M. Coxon, M. P. Hartshorn, and W. J. Rae, *Tetrahedron*, **26**, 1091 (1970).

(15) We propose the trivial name kylinane (shä lean ane) for this new carbon skeleton in honor of the Swedish phycologist Kylin, who first described *L. pacifica*.



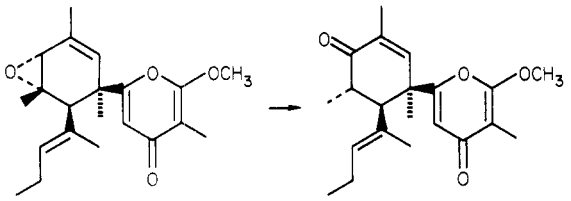
(16) This follows the IUPAC 1968 Tentative Rules, Section E, Fundamental Stereochemistry, for designating chiral centers where the relative but not absolute configuration is known (E-5.10.): *J. Org. Chem.*, **35**, 2849 (1970).

(17) B. M. Howard and W. Fenical, *Tetrahedron Lett.*, 1687 (1975).

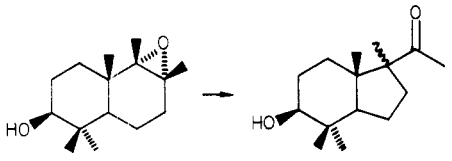
(18) C. Ireland, D. J. Faulkner, B. A. Solheim, and J. Clardy, *J. Am. Chem. Soc.*, **100**, 1002 (1978).

(19) The Lewis acid opening of 1,3-cyclohexadiene monoepoxide to 3-cyclohexenone (J. Staroscik and B. Rickborn, *J. Am. Chem. Soc.*, **93**, 3046 (1971)) illustrates that a secondary alkyl cation dominates over a secondary cation in the transition state of this reaction.

(20) Rickborn and Gerkin[21] have also reported that rearrangement of 1,2-dimethylcyclohexene oxide by $LiClO_4$ in benzene yields 1-acetyl-1-methylcyclopentane (91%) and 2,2-dimethylcyclohexane (9%).



12 13



14 15

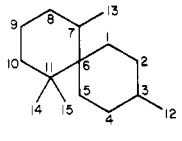
it is not clear how to predict which group will preferentially migrate (methyl vs. ring C).

Since deoxyprepacifenol (7) is a possible precursor to kylinone (1) and has been isolated from this species of algae collected at a different location,[22] we were careful to show that 1 was not an artifact. Subjecting 7 to our workup conditions showed that it was inert. In addition, treatment of 7 with *p*-toluenesulfonic acid in benzene did not yield 1, but instead a rather complex mixture of products were obtained that were not fully characterized.

The 10-halochamigrene skeleton appears to be a key intermediate in the biogenesis of many *Laurencia*-derived sesquiterpenes,[1] including cuparanes, spirolauranes, and perforanes. The biogenesis of each of these is based upon either ring contraction or expansion at the spirane junction of a 10-halochamigrene derivative. Our observation that kylinone (1) is an alkyl-rearranged chamigrene provides an interesting contrast to these former and more widely observed biogenetic possibilities.

Experimental Section

The NMR spectra were recorded on a JEOL FX-100 PFT spectrometer operating at 99.55 MHz for ^1H and 25.0 MHz for ^{13}C spectra and on a HXS 360-MHz spectrometer at Stanford University. GC/MS data were obtained on a Finnigan 4000 system equipped with a $^1/_8$ in. × 6 ft glass column packed with 3% OV-17 on Chromosorb Q and temperature programmed in the range 130–225 °C at either 5 or 10 °C min^{-1}. High-performance liquid chromatography (LC) was done on a Waters ALC 201 using Porasil A and μ-Porasil columns. Optical rotations were measured on a Jasco ORD/CD with a 0.1-dm cell (0.5 mL). All solvents were reagent grade and distilled for high-performance LC use. Low-boiling petroleum ether was used in all instances. Spectral grade solvents were used for NMR (Me_4Si standard) determinations. A previously described procedure was used to prepare 3-bromo-2-cyclohexenone.[23] The chamigrane ring is numbered as shown.



Collections and Extractions. *L. pacifica* was collected intertidally during February 1978. The seaweed was kept frozen until extracted. The alga was extracted with methanol in a Soxhlet apparatus. The dry weight of the seaweed after extraction was 1.2 kg. Silica chromatography (Grace grade 62, 60–200 mesh, activated) of the concentrated extract using petroleum ether, petroleum ether/benzene (1:1), and benzene yielded 670 mg of semipure oil. When allowed to stand, the oil yielded crystalline aplysin (2), 100 mg. High-performance LC, petroleum ether/benzene (3:1), of the remaining oil yielded the compounds below.

Kylinone (1) was obtained as a colorless oil (2 mg, 0.0002% yield) from high-performance LC (fractions 13–14): $[\alpha]^{25}_D$ +31° (*c* 1.9, CH_3OH); ^1H NMR (100 MHz, $CDCl_3$) δ 0.90 (s, CH_3), 1.14 (d, *J* = 7.1 Hz, Me_{13}), 1.20 (s, CH_3), 1.67 (s, Me_{12}), 1.80 (t, 1 H, *J* = 13 Hz, H_{5a}), 2.33 (ddd, 1 H, *J* = 13, 4, 3 Hz, H_{2e}), 2.67 (ddd, 1 H, *J* = 2.5, 4.9, 13.6 Hz, H_{5e}), 3.05 (dq, 1 H, *J* = 3.2, 7.1 Hz, H_8), 4.66 (dd, 1 H, *J* = 4.9, 12.2 Hz, H_4), 5.95 (d, 1 H, *J* = 3.2 Hz, H_9); spin decoupling yielded the following results, irradiation at δ 1.14 (Me_{13}) collapses the signal at δ 3.05 (H_8) to a doublet, irradiation at δ 2.67 (H_{5e}) collapses the signal at δ 4.66 (H_{4a}) to a doublet, irradiation at δ 3.05 collapses signals at δ 1.14 and 5.95 (H_9) to singlets, irradiation at δ 4.66 collapses the signal at δ 2.67 to a double of doublets, and irradiation at δ 5.95 collapses the signal at δ 3.05 to a quartet; MS *m/e* 410, 412, 414, 416 (3:7:5:1), 374, 376, 378 (1:2:1) (exact mass 375.9868 vs. 375.9861 calcd for $C_{15}H_{20}O^{79}Br^{81}Br$), 295, 297 (1:1) (exact mass 297.0678 vs. 297.0678 calcd for $C_{15}H_{20}O^{81}Br$), 174, 176 (1:1) (exact mass 179.0049 vs. 174.0044 calcd for $C_7H_{11}{}^{79}Br$); IR 1725 cm^{-1}; ^{13}C NMR (25 MHz, $CDCl_3$) δ 209.9 (s, C_7), 136.6 (s, C_{10}), 130.0 (d, C_9), 70.8 (s, C_3), 60.6 (d, C_4), 58.4 (s), 48.7 (s), 42.6 (d, C_8), 40.1 (t), 36.3 (t), 27.1 (t), 24.8 (q), 24.0 (q, C_{12}), 23.8 (q), 14.2 (q). The ^{13}C assignment of C_{12} was identified by an SFORD at δ 1.67 in the ^1H NMR spectrum.

Aplysin (2), mp 82–83 (lit.[3b] mp 85–86 °C), 30 mg (0.01% total yield), was obtained by high-performance LC (fraction 7). Its spectral properties, including ^1H[3b] and ^{13}C NMR[22] and mass spectrum[3b] were identical with those of an authentic sample and with those in the literature.

Debromoaplysin (3), 30 mg (0.0025%), was obtained as a clear oil by high-performance LC (fraction 4). Its ^1H NMR, mass, and IR spectra were identical with those in the literature.[3b]

Pacifenol (4), mp 148–150 °C (lit.[4] 149–150.5 °C), 50 mg (0.004%), was obtained as described below. Its ^1H, ^{13}C NMR, and mass spectra were identical with those of an authentic sample and with those in the literature.[4,22]

Pacifidiene (5), mp 110–112 °C (lit. mp 117–118.5[4] and 114–116[5] °C), 12 mg (0.001%), was obtained by high performance LC as described below. Its mass and ^1H NMR spectra were identical with those in the literature.[5]

Debromolaurinterol (6), 30 mg (0.003%), was obtained as the major component of a complex mixture by high-performance LC (fractions 9–11). Its mass and ^1H NMR spectra were identical with those in the literature.[6]

High-Pressure LC Isolation of 4 and 5. After the elution of 1 above, an additional 480 mL of solvent was collected. The column was then eluted with ethyl acetate (250 mL) to yield a mixture which included 4 and 5. The solvent was removed, and the pacifenol (4) was purified by crystallization from benzene–hexane. The mother liquor was rechromatographed by high-performance LC using $CHCl_3$–CCl_4 solvent (1:1) to yield pacifidiene (5).

Deoxyprepacifenol (7) to Kylinone (1). Deoxyprepacifenol (5 mg) in 5 mL of dry diethyl ether was added to freshly distilled BF_3·Et_2O (15 drops, 6.0 μmol) dissolved in 5 mL of dry diethyl ether and cooled under an N_2 atmosphere in a dry ice/acetone bath. This mixture was stirred and allowed to warm to room temperature over a period of 18 h. The reaction was quenched with saturated $NaHCO_3$ solution. The organic layer was removed, washed with water, dried over $MgSO_4$, and concentrated under vacuum. The residue was passed through a silica gel pad in benzene to give kylinone (4.7 mg, 94% yield), mp 143–146 °C dec. All of its spectral properties including ^{13}C, ^1H NMR, IR, and mass spectra were identical with those of 1 from *L. pacifica*.

Epoxide 8 to 9. Employing the procedure above with 5 mg of 8 and freshly distilled BF_3·Et_2O yielded 9:[17] ^1H NMR (100 MHz, $CDCl_3$) δ 6.38 (d, *J* = 10, H_{10}), 5.78 (d, *J* = 10, H_9), 4.52 (dd, *J* = 12, 4, H_4), 1.64 (s, Me_{12}), 1.25 (s, CH_3), 1.10 (d, *J* = 7, Me_{13}), 0.98 (s, CH_3); MS *m/e* 317, 319, and 321 (M^+ – Me), 303, 305, 307, 296, 298, 173, 96 (base peak).

Olefin Epoxide 10 to 11. Employing the procedure above with 5 mg of 10 and freshly distilled BF_3·Et_2O yielded 11 in quantitative yield: ^1H NMR (100 MHz, $CDCl_3$) δ 9.83 (s, H_8), 5.70 (d, *J* = 6 Hz), 5.25 (d, *J* = 6 Hz), 4.25 (dd, *J* = 13, 4 Hz, H_4), 1.66 (s, Me_{12}),

(21) B. Rickborn and R. M. Gerkin, *J. Am. Chem. Soc.*, **93**, 1693 (1971).
(22) J. J. Sims, A. F. Rose, and R. R. Izac in "Marine Natural Products—Chemical and Biological Perspectives", Vol. 2, P. J. Scheuer, Ed., Academic Press, New York, 1978, Chapter 5.
(23) E. Piers and I. Nagakura, *Synth. Commun.*, **5**, 193 (1975).

1.22 (s, CH_3), 1.00 (s, 2 CH_3); MS m/e 303, 305, and 307 (M^+ - CHO), 267, 269, 187, 145 (base peak).

Epoxide 8 to Olefin Epoxide 10. Compound 8 (5 mg) was added to freshly prepared t-BuONa/t-BuOH (2 mL). This mixture was warmed to 60 °C for 18 h, diluted with 10 mL of benzene, and dried over $MgSO_4$. The solvent was removed and the residue passed through a pad of silica gel with benzene to give 3-chloro-4-bromo-Δ^9-chamigrene 7,8-epoxide (**10**; 3.5 mg, 83% yield): ^1H NMR (100 MHz, $CDCl_3$) δ 5.64 (br s, 2 H, $H_{9,10}$), 4.59 (dd, J = 12, 4 Hz, H_4), 2.92 (br s, H_8), 1.66 (s, Me_{12}), 1.57 (s, Me_{13}), 1.07 (s, CH_3), 1.00 (s, CH_3); MS m/e 332, 334, and 336 (M^+), 289, 291, 293, 253, 255, 173, 98 (base peak).

Acid Treatment of Deoxyprepacifenol 7. Deoxyprepacifenol (1.0 mg) was dissolved in 5 mL of dry benzene with a single crystal of p-toluenesulfonic acid and the mixture stirred for 18 h at room temperature. After neutralization with saturated $NaHCO_3$ solution, the organic layer was dried over $MgSO_4$, and the solvent was removed. The residue was passed through a silica gel pad with benzene. The product was analyzed by GC/MS and ^1H NMR, and no kylinone or deoxyprepacifenol was detected.

Silica Treatment of Deoxyprepacifenol 7. Deoxyprepacifenol (1.0 mg) was dissolved in 5 mL of chloroform with activated silica (0.5 g) and the mixture stirred at room temperature for 24 h. The silica was removed by filtration to give deoxyprepacifenol, identical with the starting material.

Acknowledgment. We thank Professor L. Goff (UCSC) for guidance in alga identification. We also thank the NOAA office of Sea Grant for support of this work and for a traineeship to S.J.S. and the NSF Chemical Instrumentation Program for their financial assistance in the purchase of a GC/MS and PFT NMR apparatus. We are also grateful to Mr. I. Papiashvili for his technical assistance with the GC/MS work, to Dr. W. Fenical (UCSD) for a sample of **8**, and to Professor J. J. Sims (UCR) for a sample of **7**. The exact mass measurements were obtained by Ms. Sheri Ogden at the UCB Mass Spectrometry Laboratory, and the 360-MHz NMR spectra were obtained at the Stanford Magnetic Resonance Laboratory supported by the NSF (Grant No. GR 23633) and the NIH (Grant No. RR00711).

Registry No. **1**, 72036-07-8; **2**, 6790-63-2; **3**, 23444-68-0; **4**, 33880-90-9; **5**, 33880-92-1; **6**, 10539-88-5; **7**, 58967-05-8; **8**, 57685-66-2; **9**, 57685-68-4; **10**, 72036-08-9; **11**, 72036-09-0.

Active Esters of 9-Fluorenylmethyloxycarbonyl Amino Acids and Their Application in the Stepwise Lengthening of a Peptide Chain

Agnes Bodanszky, Miklos Bodanszky,* Nagarajan Chandramouli, Joseph Z. Kwei, Jean Martinez, and John C. Tolle

Department of Chemistry, Case Western Reserve University, Cleveland, Ohio 44106

Received July 20, 1979

Preparation and properties of p-nitrophenyl esters of several 9-fluorenylmethyloxycarbonyl (Fmoc) amino acids are described. The Fmoc derivatives of the hindered amino acids valine and isoleucine were converted to the more reactive o-nitrophenyl esters while N-Fmoc-O-benzyl-L-tyrosine was esterified with pentachlorophenol. In the selection of experimental conditions for coupling reactions an effort was made to keep premature cleavage of the Fmoc group at a minimum. As an example for chain lengthening with Fmoc amino acid active esters the preparation of the C-terminal 7-peptide segment of chicken VIP is described.

The 9-fluorenylmethyloxycarbonyl (Fmoc) group was proposed by Carpino and Han[1] for the protection of the α-amino function in peptide synthesis. A particular advantage of the new protecting group is that it can be removed by basic reagents under mild conditions and yet is quite resistant to acidolysis.[2] These properties of the Fmoc group allow the use of acid-labile protecting groups for the blocking of the C-terminal carboxyl and various side-chain functions. Thus, final deprotection can be carried out with relatively weak acids, again under mild conditions. The Fmoc protection has already been applied, and with considerable success, in solid-phase syntheses of biologically active peptides.[3,4] We report here our experiments on stepwise chain lengthening[5] *in solution*, with nitrophenyl esters[6] of Fmoc amino acids[7] as acylating agents. In this process premature deprotection could be caused by the action of the amino component. Indeed, a moderate loss of Fmoc groups during coupling was experienced[8] in our laboratory, but we expected that under carefully chosen reaction conditions this side reaction can be kept at an acceptable minimum. Therefore, the acylations with active esters of Fmoc amino acids were catalyzed with 1-hydroxybenzotriazole (HOBt).[9] This acidic additive diminished the basicity of the reaction mixture and considerably reduced the time during which the Fmoc derivatives were exposed to the action of the nucleophilic component.

For removal of the Fmoc group the use of diethylamine[1] was also explored. The reagent is more easily removed from the reaction mixtures and less readily forms a tertiary amine by addition to dibenzofulvene (the byproduct of deprotection) than the recommended[1] and somewhat more efficient piperidine. The polymeric derivative of piperazine proposed[10] for the removal of the Fmoc group was not

(1) L. A. Carpino and G. Y. Han, *J. Am. Chem. Soc.*, **92**, 5748 (1970); *J. Org. Chem.* **37**, 3404 (1972); *ibid.*, **38**, 4218 (1973).

(2) The originally observed[1] resistance of the Fmoc group to catalytic hydrogenation could not be confirmed in our laboratory: J. Martinez, J. C. Tolle, and M. Bodanszky, *J. Org. Chem.*, **44**, 3596 (1979).

(3) E. Atherton, H. Fox, D. Harkiss, C. J. Logan, R. C. Sheppard, and B. J. Williams, *J. Chem. Soc., Chem. Commun.*, 537 (1978); E. Atherton, H. Fox, D. Harkiss, and R. C. Sheppard, *ibid.*, 539 (1978).

(4) C. D. Chang and J. Meienhofer, *Int. J. Pept. Protein Res.*, 11, 246 (1978).

(5) M. Bodanszky, *Ann. N.Y. Acad. Sci.*, **88**, 655 (1960).

(6) M. Bodanszky, *Nature (London)*, **15**, 685 (1955).

(7) The active esters Fmoc-glycine N-hydroxysuccinimide ester and Fmoc-glycine 8-hydroxyquinoline ester were described in ref 1. The p-nitrophenyl ester of Fmoc-L-asparagine was used in solid-phase syntheses[3,4] but its properties were not reported.

(8) M. Bodanszky, S. S. Deshmane, and J. Martinez, *J. Org. Chem.*, **44**, 1622 (1979).

(9) W. König and R. Geiger, *Chem. Ber.*, **106**, 3626 (1973).

0022-3263/80/1945-0072$01.00/0