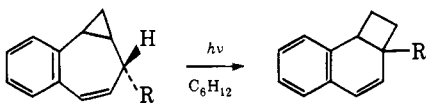
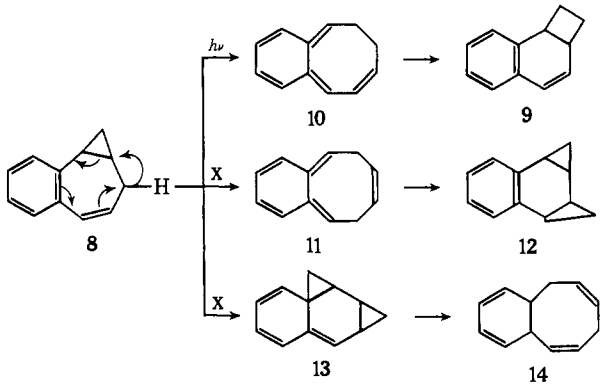


8a, R = H
b, R = CH_3

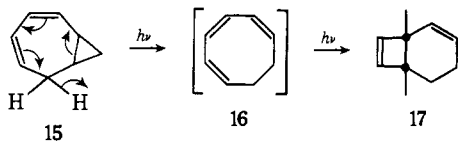
course of this isomerization, the methyl derivative **9b** was studied. Irradiation of **9b** under similar conditions as the parent compound yielded one major product as detected by vpc. The mass spectrum indicated that the product was isomeric with **8b**, and the base peak of 142 (P − 28) was strongly suggestive that a simple cyclobutane ring was present. The 100-MHz nmr (CCl_4) spectrum showed: τ 3.12 (m, 4 H), 3.78 (d, J = 10 Hz, 1 H), 4.46 (d, J = 10 Hz, 1 H), 6.84 (br t, 1 H), 7.98 (m, 4 H), and 8.86 (s, 3 H). The appearance of the two vinyl protons as a simple AB quartet and the methyl group as a sharp singlet indicates that both the vinyl and methyl groups are bonded to tertiary centers. This, together with the mass spectral evidence, establishes the photoproduct as **9b**.

The formation of **9a** from **8a** is explained by a homo-1,7 shift in excited **8a** yielding **10** which undergoes valence tautomerization to **9a**. Neither **12**, which would have been expected from hydrogen shift of **1a** in the opposite sense, nor **14**, which would have been formed



via the homo-1,5-sigmatropic shift route, could be detected in these studies. The exclusive formation of the hydrogen-migrated product **9b** from **8b** is in accord with the much greater migratory aptitude of hydrogen *vs.* methyl in the benzotropilidene system.[8] While qualitative observations from preparative irradiation suggested a moderately efficient process, quantum yields were measured to directly compare this reaction with hydrogen migration in the parent 3,4-benzotropilidene. The quantum yields for disappearance of **8a** and appearance of **9a** were 0.24 and 0.20, respectively, indicating a process of good efficiency. Since the quantum yield for hydrogen migration in 3,4-benzotropilidene is 0.87,[2b] the analogous shift in the homo-system is four–five times less efficient.

The present results demonstrate the unique photochemical behavior of 1,2-homo-3,4-benzotropilidenes. Previous studies on homotropone[9] and homoazepin[10] derivatives showed that the major products were those expected from the individual chromophores without specific involvement of the total homo-π system. The previous reported irradiation of the homotropilidene **15** to afford **17**, which was noted without comment concerning mechanism,[11] may also proceed *via* a homo-1,7-hydrogen shift. The observed product **17** would necessarily have arisen by secondary irradiation of initially formed **16**. The present results, together with



the inferences drawn for the literature, indicate the unique involvement of the cyclopropane ring of homotropilidenes and homobenzotropilidenes in the highly selective and moderately efficient homo-1,7 shifts. Full synthetic details and mechanistic discussion will be presented in our full paper.[12]

(8) Photolysis of 7-methyl-3,4-benzotropilidene proceeds with a quantum efficiency of 0.93 and yields solely 5-methylbenzonorcaradiene. The migration ratio of hydrogen *vs.* methyl is at least 1000:1.

(9) L. A. Paquette and R. J. Haluska, *J. Org. Chem.*, **35**, 132 (1970).

(10) L. A. Paquette and O. Cox, *J. Amer. Chem. Soc.*, **89**, 5633 (1967).

(11) (a) W. R. Roth and B. Peltzer, *Justus Liebigs Ann. Chem.*, **685**, 56 (1965). (b) Interestingly, labeling studies used to support a 1,5-hydrogen shift mechanism in the photochemistry of 1,3,6-cyclooctatriene are equally consistent with the sequence

$$\text{1,3,6-cyclooctatriene} \xrightarrow{h\nu} \mathbf{15} \xrightarrow{h\nu} \mathbf{16} \xrightarrow{h\nu} \mathbf{17}$$

(12) All new compounds gave acceptable combustion analyses. The general route to the homobenzotropilidenes involves Simmons–Smith reaction with the corresponding 3,4-benzocycloheptа-1,3-diene, followed by NBS bromination and dehydrobromination with $CaHPO_3$ in DMF (D. J. Bertelli and C. C. Ong, *J. Amer. Chem. Soc.*, **87**, 3719 (1965)). Direct Simmons–Smith reaction with 3,4-benzotropilidenes gives complex mixtures of products.

(13) Alfred P. Sloan Fellow (1970–1972); Camille and Henry Dreyfus Teacher–Scholar Awardee, 1971–1976.

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Nybomycin. VI. Incorporation of Acetate-^{13}C, Acetate-^{14}C, and Methionine-^{14}C[1]

Sir:

The structure **1** assigned[2] to the antibiotic nybomycin by its spectral and chemical properties has been confirmed by the total synthesis[1] of nybomycin. The unusual structure of nybomycin involves a ring system thus far unique in nature (except for the naturally occurring deoxynybomycin)[3,4] and it stimulates speculation on its origin.[5] We present evidence here, including studies with a ^{13}C label, which clearly and unequivocally defines acetate as the source of the exterior carbons of the pyridone rings but rules out acetate as the primary source of the carbons of the central ring. We also present evidence which defines

(1) Paper V: R. M. Forbis and K. L. Rinehart, Jr., *J. Antibiot.*, 24, 326 (1971).

(2) K. L. Rinehart, Jr., G. Leadbetter, R. A. Larson, and R. M. Forbis, *J. Amer. Chem. Soc.*, 92, 6994 (1970).

(3) H. Naganawa, T. Wakashiro, A. Yagi, S. Kondo, T. Takita, M. Hamada, K. Maeda, and H. Umezawa, *J. Antibiot.*, 23, 365 (1970).

(4) R. M. Forbis and K. L. Rinehart, Jr., *J. Amer. Chem. Soc.*, 92, 6995 (1970).

(5) (a) K. L. Rinehart, Jr., 17th National Organic Chemistry Symposium, Bloomington, Ind., June 25–29, 1961; (b) K. L. Rinehart, Jr., R. A. Larson, R. M. Forbis, and G. Leadbetter, Abstracts, 5th International Symposium on the Chemistry of Natural Products, IUPAC, London, July 1968, p 79; (c) R. A. Larson, Ph.D. Thesis, University of Illinois, Urbana, 1968.

Table I. Incorporation of Labeled Precursors into Nybomycin

	Methionine-*methyl*-^{14}C	Sodium acetate-*2*-^{14}C	Sodium acetate-*1*-^{14}C	Sodium acetate-*1*-^{13}C
Precursor added				
Amount	0.295 mg	0.405 mg	0.388 mg	5.80 g
Label	14.9 mCi/mmol	50.6 mCi/mmol	52.9 mCi/mmol	90% ^{13}C
Nybomycin isolated				
Amount	74 mg	59 mg	63 mg	61 mg[a]
Label	16.12 μCi/mmol	0.732 μCi/mmol	1.159 μCi/mmol	46% ^{13}C[b]
% incorporation	13.6	0.058	0.098	0.59[c]
Dilution	920	69,130	45,640	1.96[b]
% of total incorporation				
C-2	52.3	0.0	0.0	
C-11′	47.7	0.0	0.0	
C-6	0.0	2.2	23.8	25[d]
C-8	0.0	2.2	19.5	18[d]
C-6′	0.0	15.5	0.2	
C-8′	0.0	11.9	0.2	
C-4	0.0			26[d]
C-10	0.0			30[d]

[a] Based on the amount of nybomycin butyrate (**8**) isolated (55 mg) and the yield in conversion of **1** to **8** (74%). [b] Based on per cent label at each labeled carbon estimated from mass spectra of nybomycin butyrate (**8**). [c] Based on four labeled carbons (each 46%) per nybomycin and 61 mg of nybomycin. [d] Based on peak integrals in an undecoupled carbon magnetic resonance spectrum.

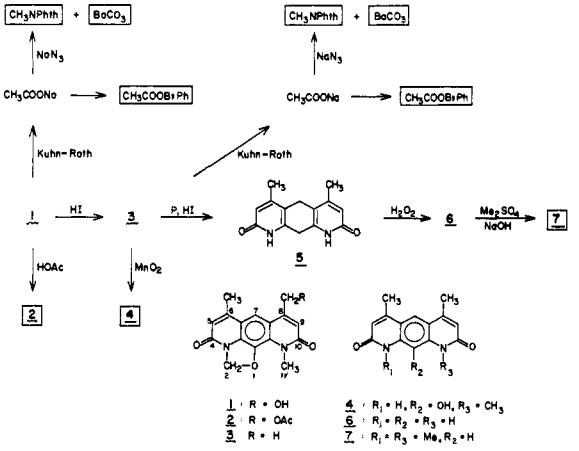

Figure 1. Numbering system employed and degradation scheme for location of the ^{14}C label in nybomycin (**1**); primary reference compounds for counting are enclosed in boxes: Phth = phthaloyl; BrPh = *p*-bromophenacyl.

methionine as the source of the one-carbon *N*-methyl and *N*–CH_2O units.

Streptomyces sp. D-57 was grown as described earlier,[6] with ^{14}C-labeled precursors added (after 8–10 days) as shown in Table I. After 15–18 days' growth the nybomycin produced was isolated and purified by the usual method.[7] Carbon-14 label was located by the degradation scheme shown in Figure 1.

From these results it is clear that methionine is well incorporated and labels only C-2 and C-11′ (numbering system in Figure 1). It is also clear that acetate is well incorporated and labels C-6′ and C-8′ (from acetate methyl) as well as C-6 and C-8 (from acetate carboxyl). The amount of label incorporated by acetate-*1*-^{14}C at C-6 and C-8 at each position is approximately one-fourth of the total for nybomycin. Slightly lower percentages are specifically incorporated at C-6′ and C-8′ from acetate-*2*-^{14}C, as expected for the biosynthetically more mobile[8] methyl group of acetate. This implies (but does not establish) that the remaining exterior carbons (C-4, C-5, C-9, C-10) of the pyridone rings may also be labeled by acetate to the same extent. Existing degradation schemes for nybomycin do not, however, allow the testing of that hypothesis for ^{14}C-labeled nybomycin; consequently, we have turned to studies with the ^{13}C label.

Sodium acetate-*1*-^{13}C was added after 6 days to the medium for *Streptomyces* sp. D-57 and the labeled nybomycin was isolated as reported above and converted to its butyrate **8**.[2] Isotope ratio mass spectra (flat top peaks) of the labeled butyrate indicated 41% unlabeled nybomycin, 6% monolabeled, 11% dilabeled, 17% trilabeled, and 22% tetralabeled, plus 4% pentalabeled. (This corresponds to an average label of 46% (Table I) at each of the four carbons labeled (*vide sequitor*).) The ^{13}C nmr spectrum of unlabeled nybomycin butyrate (**8**) showed peaks for the expected 20 carbon atoms, which were assigned (Table II) with the help of standard chemical-shift data,[9] off-resonance

Table II. Carbon Magnetic Resonance Peaks for Nybomycin Butyrate (**8**)[a]

Carbon atom	Absorption, ppm[b]	Carbon atom	Absorption, ppm[b]
C-2	85.9	C-9	121.5
C-4	158.1	C-10	161.3
C-5	118.6	C-11a	135.5
C-6	143.7	C-11′	32.6
C-6′	17.8	C-12	125.6
C-6a	113.4	C-12a	132.2
C-7	112.0	C-1′	172.7
C-7a	117.4	C-2′	36.0
C-8	147.1	C-3′	18.4
C-8′	61.9	C-4′	13.7

[a] Numbering system for **8** is shown in Figure 1. [b] From TMS; $CDCl_3$ = 76.9 ppm.

(6) T. D. Brock and W. T. Sokolski, *Antibiot. Chemother.* (*Washington, D. C.*), **8**, 631 (1958).

(7) T. E. Eble, G. A. Boyack, C. M. Large, and W. H. DeVries, *Antibiot. Chemother.* (*Washington, D. C.*), **8**, 627 (1958).

(8) J. H. Richards and J. B. Hendrickson, "The Biosynthesis of Steroids, Terpenes and Acetogenins," W. A. Benjamin, New York, N. Y., 1964, p 142.

(9) G. C. Levy and G. L. Nelson, "Carbon-13 Nuclear Magnetic Resonance for Organic Chemists," Wiley-Interscience, New York, N. Y., 1972.

proton decoupling, ^{13}C–^{1}H splitting patterns, and consideration of the effects manifest in the proton magnetic resonance spectrum of **8**.[2]

The ^{13}C magnetic resonance spectrum of ^{13}C-labeled nybomycin butyrate showed four enormously enriched peaks of roughly equal intensity for C-4, C-6, C-8, and C-10 and no observable enrichment at any other carbons. Thus, the proposition arrived at from the ^{14}C data—of incorporation of four acetate units into the exterior carbons of the pyridone rings—is substantiated. More importantly, the present results establish that the carbon atoms of the central ring *do not* come from acetate *via* a phloroglucinol-type pathway.[10] The origin of those carbon atoms will be described in future reports.

Acknowledgments. This work was supported by Public Health Service Grants AI 01278 and AI 04769 from the National Institute of Allergy and Infectious Diseases, and Grant CA 12388 from the National Cancer Institute. We thank Mr. Robert Thrift for the ^{13}C nmr spectra, Mr. Joseph Wrona for the isotope ratio mass spectra, and Dr. Robert F. Nystrom for assistance with the synthesis of ^{13}C-labeled acetate.

(10) J. D. Bu'Lock, "The Biosynthesis of Natural Products," McGraw-Hill, London, 1965, p 88.

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Received December 27, 1972

Structures of Stemodin and Stemodinone

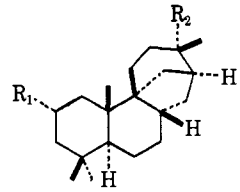
Sir:

Examination of leaf constituents of the rare littoral plant *Stemodia maritima* L. (Scrophulariaceae),[1] obtained from the Palisadoes peninsula of Jamaica, has brought to light two new diterpenes containing an unusual tetracyclic skeleton. Structural elucidation of these substances is reported herein.

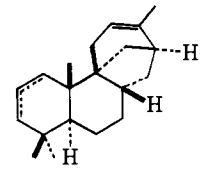
Stemodin (**1**), $C_{20}H_{34}O_2$,[2] mp 196–197°, $[\alpha]D$ −2.6° (*c* 1.07, pyridine), shows hydroxyl (3340, 3220 cm^{-1}) but no carbonyl absorption in its infrared spectrum. The presence of two OH groups is deduced from 1 H singlets in the nmr spectrum (DMSO-d_6) of **1** at δ 3.23 and 3.79; in addition the spectrum (in $CDCl_3$) revealed four methyl groups at δ 0.90, 0.93, 0.97, and 1.08 and a proton (δ 3.71, t of t, J = 11, 3.5 Hz) attached to carbon bearing a hydroxyl group and flanked by two methylene groups.[3] From the coupling constants this proton is axial, and hence the hydroxyl group must be equatorial.

Acetylation of stemodin (acetic anhydride in pyridine at 50° for 6 hr) yielded a monoacetate **2**, mp 141–142°, $[\alpha]D$ −30.0° (*c* 1.19, $CHCl_3$), ν 3620, 3460, and 1730 cm^{-1}, δ 2.02 (3 H, s), 4.87 (1 H, t of t, J = 11, 3.5 Hz), confirming the presence of secondary and tertiary OH groups in **1**.

Dehydration of stemodin (phosphorus oxychloride in pyridine at 65° for 1.5 hr) afforded an oily hydrocarbon **3**, $C_{20}H_{30}$, which contained a vinylic methyl group (δ 1.60, d, J = 2 Hz) and three olefinic protons (δ 5.22, 2 H, m, and δ 5.00 1 H, broad). Hydrogenation of this substance over 5% Pd/C at atmospheric pressure resulted in the uptake of 2 equiv of hydrogen with the formation of a saturated derivative **4**, $C_{20}H_{34}$, $[\alpha]D$ +10.1° (*c* 1.13, $CHCl_3$).[4]



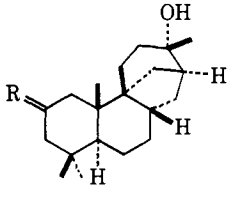
1, $R_1 = R_2 = OH$
2, $R_1 = OCOCH_3$; $R_2 = OH$
4, $R_1 = R_2 = H$



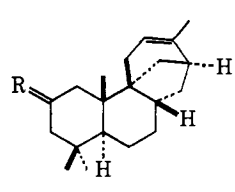
3

Stemodinone (**5**), $C_{20}H_{32}O_2$, mp 215–216°, $[\alpha]D$ +14.3° (*c* 1.00, $CHCl_3$), was isolated from a less polar fraction of *S. Maritima* and showed carbonyl (1700 cm^{-1}) as well as hydroxyl (3600, 3460 cm^{-1}) absorption in its infrared spectrum. Its relationship with stemodin was established by oxidation of the latter with Jones reagent, which gave **5** in good yield. The Raman spectrum of **5** showed no lines due to C=C functionality; this, in conjunction with the failure to observe hydrogen uptake, provided evidence for the tetracyclic nature of the stemodin skeleton. Treatment of stemodinone with $POCl_3$ in pyridine (65°, 1.5 hr) afforded the dehydro compound **6**, mp 93–95°, which showed carbonyl (1700 cm^{-1}) but no hydroxyl absorption. The ultraviolet spectrum of this compound ruled out an α,β-unsaturated ketone, and the nmr spectrum revealed a single olefinic proton (δ 5.00) which was coupled to vicinal methylene hydrogens. This latter feature, taken with the foregoing evidence, rendered a structural compromise between the stemodins and the familiar tetracyclic diterpene skeletons[5] unlikely.

Reduction of stemodinone (**5**) under Huang-Minlon conditions[6] furnished desoxy derivative **7**, $C_{20}H_{34}O$, mp 143–144°, $[\alpha]D$ +6.2° (*c* 1.02, $CHCl_3$), containing a tertiary methyl carbinol (δ 1.13, 3 H, s, and ir absorption at 3590 and 3430 cm^{-1}). Dehydration of **7** (phosphorus oxychloride in pyridine) gave a crystalline olefin **8**, $C_{20}H_{32}$, mp 52–53°, $[\alpha]D$ +36.6° (*c* 1.00, $CHCl_3$), which displayed the anticipated broad olefinic proton (δ 5.00) and vinylic CH_3 (δ 1.60, d, J = 2 Hz) in the nmr spectrum.



5, R = O
7, $R = H_2$



6, R = O
8, $R = H_2$

Structural elucidation of the novel stemodin ring

(1) C. D. Adams, "Flowering Plants of Jamaica," University of the West Indies Press, Mona, Jamaica, 1972, p 662.
(2) Elemental analyses and mass spectra were obtained in agreement with all compositions shown.
(3) C. M. Chen and T. Murakami, *Tetrahedron Lett.*, 1121 (1971).
(4) Addition of hydrogen is presumed to occur from the less hindered side of the bicyclo[3.2.1]octane system, resulting in the stereochemistry shown.
(5) R. McCrindle and K. H. Overton, *Advan. Org. Chem.*, **5**, 47 (1965).
(6) H. O. House, "Modern Synthetic Reactions," 2nd ed, W. A. Benjamin, Menlo Park, Calif., 1972, p 228.