Table I. Incorporation Experiments with ^{13}C-Labeled Formamide 5 and Isothiocyanate 7

precursor	amount embedded	incubation period	weight of animal	PN^{13}C (6), m/z ^{13}C experiment 232/231	^{13}C experiment 217/216	control 232/231	control 217/216
5	(1) 30 mg (2) 30 mg	7 days	150 g	12/100[a]	15/100[a]	13/100	16/100
7	45 mg	15 days	108 g	13/100	16/100	13/100	16/100

[a] m/z 231 and m/z 216 are arbitrarily assigned 100% intensity.

Table II. Incorporation of [^{13}C]Formate

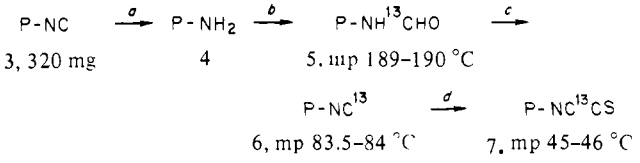
amount embedded	incubation period	wet wt animal	PN^{13}C (6), m/z experiment 232/231	experiment 217/216	control 232/231	control 217/216
(1) 102 mg (2) 102 mg	7 days 7 days	150 g 150 g	12/100	16/100	13/100	16/100

Table III. Internal distribution of C^{13}-Labeled Isocyanopupukeanane (6)[a]

sect. no.	m/z, experiment 232/231	m/z, experiment 217/216	m/z, control 232/231	m/z, control 217/216
1	15/100	15/100		
2[b]	28/100	39/100		
3	14/100	17/100		
4[b]	34/100	50/100	13/100	16/100
5	10/100	17/100		
6	12/100	16/100		
7	12/100	15/100		

[a] Approximate wet wt of animal 260 g; incubation time 14 days; wt of labeled precursor 2 × 45 mg. [b] These are the sections where the labeled precursor was imbedded.

Scheme I



[a] 6 N HCl (50 mL), reflux 4 h; 30% NaOH; distill; 140 mg (69%). [b] $H^{13}CO_2H$ (91.7% ^{13}C, Prochem), 103 mg + 117 mg 4, sealed tube, 110 °C, 10 h; 85 mg (64%). [c] 5 (497 mg) in CH_2Cl_2 (15 mL) and pyridine (1.1 mL) at 0 °C, $POCl_3$ (700 mg), 20 min at 0 °C; after 2 cycles 193 mg of 6 (42%); ν_{max} (KBr) 2099 cm^{-1} vs. PN^{12}C at 2137 cm^{-1}. [d] 6 (25 mg), S (5 mg), sealed tube, 110 °C, 16 h; after workup, 5.6 mg (18%); ν_{max} (KBr) 2166, 2101 cm^{-1}.

7 and [^{13}C]formate the whole animals were worked up.

The results are summarized in Tables I and II. The data show unambiguously that no formamide **5** or isothiocyanate **7** is transformed into labeled 2-isocyanopupukeanane (**6**)[10] (Table I) and that, in analogy with the *Penicillium* research,[2] formate is not utilized by the sponge for isocyano biosynthesis (Table II).

In the experiment with labeled 2-isocyanopupukeanane (**6**), we checked diffusion of the label by analyzing seven parallel slices. The data in Table III show that little transport of label takes place in a 2-week period. Examination of the formamide $(M^+ + 1)/M^+$ (m/z 250/249) and isothiocyanate $[(M^+ + 1)/M^+$ (264/263), $(M^+ + 1 - HS)/(M^+ - HS)$ (231/230)] peaks demonstrates unequivocally that the isocyano function is the precursor of formamide and isothiocyanate in *Hymeniacidon* sp. (Table IV). The recent demonstration by Herbert and Mann[12] that the *N*-formyl carbon in the *Streptomyces* metabolite tuberin (**8**) is biosynthesized from glycine is interesting but irrelevant in this case since we have shown that *Hymeniacidon* does not transform the *N*-formyl into the isocyano function.

Table IV. Transformation of ^{13}C-Labeled 2-Isocyanopupukeanane (6) into Formamide 5 and Isothiocyanate 7[a]

sect. no.	m/z formamide (5) 250/249	m/z isothiocyanate (7) 264/263	m/z isothiocyanate (7) 231/230
1	12/100	ND[b]	20/230
2	44/100	25/100	28/100
3	18/100	ND	15/100
4	41/100	31/100	28/100
5	ND[b]	ND	17/100
6	13/100	ND	15/100
7	ND	ND	13/100

[a] See Table III for experimental parameters. [b] ND denotes <0.8% peak enhancement.

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Isolation, Characterization, and Rearrangement of *cis*- and *trans*-*N*-Acetyl-2-amino-5,6-dimethoxy-5-methylcyclohexa-1,3-diene. Models for the Proposed Precursors of Meta-Substituted Products from Carcinogenic Aromatic Amines

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Reactions of activated derivatives of the potent carcinogen *N*-acetoxy-2-acetamidofluorene (**1**) with in vitro nucleophiles have been reported by Scribner[2] and others[3,4] to produce products

(1) Deceased September 2, 1983.
(2) Scribner, J. D. *J. Am. Chem. Soc.* **1977**, *99*, 7383.
(3) Meerman, J. H. N.; Beland, F. A.; Ketterer, B.; Srai, S. K. S.; Bruins, A. P.; Mulder, G. J. *Chem.-Biol. Interact.* **1982**, *39*, 149.
(4) For related studies on derivatives of 4-ethoxyacetanilide (phenacetin), see: Hinson, J. A.; Nelson, S. D.; Gillette, J. R. *Mol. Pharmacol.* **1979**, *15*, 419. Gemborys, M. W.; Mudge, G. H.; Gribble, G. W. *J. Med. Chem.* **1980**, *23*, 304. Calder, I. C.; Creek, M. J.; Williams, P. J. *Chem.-Biol. Interact.* **1974**, *8*, 87. Calder, I. C.; Creek, M. J. *Aust. J. Chem.* **1976**, *29*, 1801. Calder, I. C.; Caciolli, S. *Ibid.* **1979**, *32*, 130. Calder, I. C.; Creek, M. J.; Williams, P. J.; Funder, C. C.; Green, C. R.; Ham, K. N.; Tange, J. D. *J. Med. Chem.* **1973**, *16*, 499.

(12) Herbert, R. B.; Mann, J. *J. Chem. Soc., Chem. Commun.* **1983**, 1008-1010.

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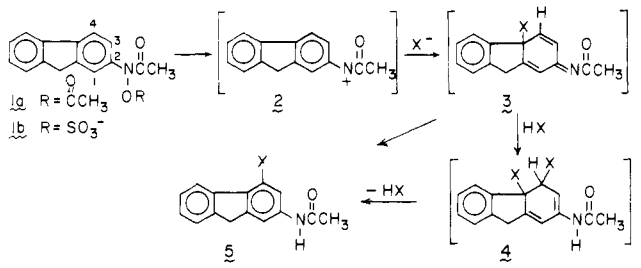
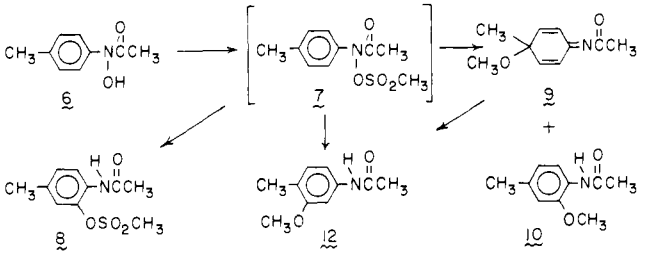
resulting from formal addition of the nucleophiles to the 4-position of the substrate. Initial attack para to the 2-acetamido moiety, via the intermediate nitrenium ion, **2**, was postulated to produce the dienone imine, **3**.[2,3] Scribner has suggested[2] that **3** is converted into **4** (X = OH), which subsequently loses water to produce **5**,

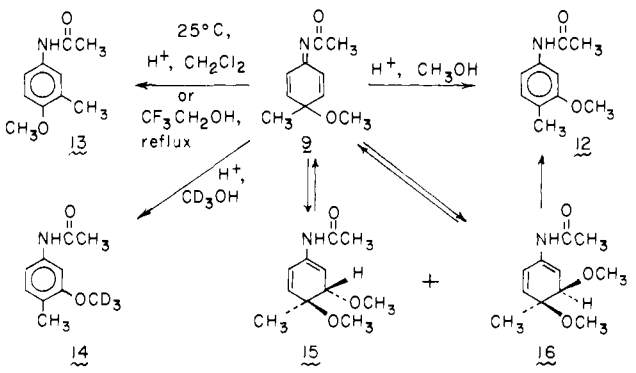


while others have hypothesized[3] that a direct 1,2-shift occurs to yield **5** (X = glutathion-*S*-yl). Although neither **3** nor **4** has been isolated, their existence has been justified on the basis of theoretical calculations.[5] We now wish to report the synthesis and characterization of simple analogues of **3** and **4** which provide a solid mechanistic basis for the **1** → **2** → **3** → **4** → **5** mechanistic path.

Treatment of **6**[6] with methanesulfonyl chloride (1.1 equiv) and triethylamine (2.5 equiv) in methylene chloride at −78 °C gave **7**.[7,8] On warming to −55 °C in nonnucleophilic solvents, **7** isomerized cleanly to **8**. However, when a solution of **7** at −78



°C was added to refluxing methanol, a mixture of 19% of **8**, 52% of **9**, and 4% of **10** was obtained.[9-11] When a similar experiment was carried out with 1.1 equiv of triethylamine, a mixture of 20% of **8** and 45% of **12**[12] was obtained. In a separate set of experiments, **12** was shown to be a secondary product derived from **9**, since treatment of **9** with methanol containing catalytic amounts of methanesulfonic acid gave only **12**. In contrast, treatment of **9** with catalytic amounts of methanesulfonic acid in methylene chloride at 25 °C gave **13**, which was the same product obtained



when **9** was heated in refluxing 2,2,2-trifluoroethanol in the absence of acid.

A major question that remained to be answered was whether the conversion of **9** into **12** involved a 1,2-shift (as in the formation of **13**) or an addition–elimination sequence as proposed by Scribner.[2] Preliminary indications, which favored the latter possiblity, were obained when **9** was converted into **14** in methanol-d_4 containing methanesulfonic acid (followed by aqueous workup).

In order to answer the mechanistic question in a definitive manner, an attempt was made to trap the possible intermediates, **15** and **16**. To a vigorously stirred solution of 1.6 g (8.9 mmol) of **9** in 50 mL of dry methanol at −2 °C was added to a solution of 0.4 mmol of methanesulfonic acid in 1.0 mL of methanol. After 35 s, the reaction was quenched by the addition of 2.0 g (19.8 mmol) of triethylamine. Nonaqueous workup gave **9** and **12**. In addition, 17% of **15**[14] and 22% of **16**[15] were isolated and characterized. The isolation of **15** and **16** illustrates that methanol can add to **9** in a Michael fashion very rapidly under mild conditions. On treatment with methanesulfonic acid in methanol both **15** and **16** rapidly gave **12** as the only product (as indicated by an NMR study of the conversion). It should be noted that the direct conversion of **16** to **12** involves an anti elimination, whereas the direct conversion of **15** to **12** involves a syn elimination. A study of the elimination of methanol from **15** and **16** at −40 °C by ^{1}H NMR spectroscopy revealed that **15**, **16**, and **9** were at least partially equilibrated prior to complete conversion to **12**. In addition, **15** reacted at a rate significantly slower than **16**. Finally, starting with pure **15**, only small amounts of **9** and **16** were detected in the acid-catalyzed formation of **12**. These data are consistent with **16** being the crucial intermediate that serves as a precursor of **12**.

In summary, the isolation of **9**, **15**, and **16**, coupled with our study of their chemical reactivity provides an excellent mechanistic model that supports Scribner's hypothesis concering the conversion of **1a** into **5** via **2**, **3**, and **4**.

Acknowledgment. We are indebted to the National Cancer Institute of the Public Health Service for a grant that supported this investigation.

Registry No. 6, 27541-21-4; **7**, 89345-78-8; **8**, 89345-79-9; **9**, 89345-80-2; **10**, 89345-81-3; **1i**, 51307-87-0; **13**, 31910-25-5; **15**, 89345-82-4; **16**, 89345-83-5.

(5) Ford, G. P.; Scribner, J. D. *J. Am. Chem. Soc.* **1981**, *103*, 4281.

(6) The preparation of **6** involved the treatment of *N*-(4-methylphenyl)-hydroxylamine with ethereal acetyl chloride in combination with a second (aqueous) phase containing sodium bicarbonate at 0 °C. For a previous report of this compound, see: Faddeeva, V. K.; Svirskaya, P. I.; Baskakov, Y. A. *Zh. Org. Khim.* **1970**, *6*, 285.

(7) Satisfactory elemental analyses and/or exact mass molecular weights have been obtained on all new compounds except **7**, which was too unstable for such characterization. All new compounds had spectral properties consistent with the assigned structure.

(8) The use of acetone-d_6 as solvent instead of methylene chloride allowed the ^{1}H NMR spectrum of **7** to be recorded at −78 °C: δ 7.16 (4 H, AB q), 3.13 (3 H, s), 2.10 (3 H, s), 1.64 (3 H, s). On warming to −55 °C, **7** rearranged to **8**: ^{1}H NMR δ 7.9–6.9 (4 H, complex m, includes NH), 3.26 (3 H, s), 2.23 (3 H, s), 2.07 (3 H, s); mp 129–130 °C.

(9) In addition, 3% of the hydrolysis product of **9**, 4-methyl-4-methoxycyclohexa-2,5-dienone (**11**) was obtained.

(10) The spectral properties of **9**: ^{1}H NMR ($CDCl_3$) δ 6.40 (4 H, AB q, J = 11 Hz), 3.11 (3 H, s), 2.20 (3 H, s), 1.37 (3 H, s); ^{13}C NMR ($CDCl_3$) δ 185.78 (s), 151.79 (s), 146.57 (d), 125.81 (d), 71.98 (s), 52.50 (q), 26.31 (q), 25.10 (q); IR (neat) 1680, 1650 cm^{-1}. Conversion of **9** into **11** occurred rapidly in aqueous acid.

(11) Compounds **8**, **9**, and **10** were not interconvertible under the reaction conditions.

(12) The melting point of **12** was 133–134 °C (lit[13] mp 132 °C). Since spectral data did not permit **12** to be distinguished from certain other possible isomers, the structure was confirmed by a single-crystal X-ray analysis. Details will be provided in a full paper on this subject.

(13) Ullmann, F.; Fitzenkam, R. *Chem. Ber.* **1905**, *39*, 3787. Friedlander, P. *Ibid.* **1916**, *49*, 955.

(14) Compound **15** was a liquid: ^{1}H NMR ($CDCl_3$) δ 7.71 (1 H, br s, NH), 6.25 (1 H, br d, J = 4 Hz), 6.15–5.64 (2 H, m), 4.28 (1 H, d, J = 4 Hz), 3.40 (3 H, s), 3.26 (3 H, s), 2.03 (3 H, s), 1.25 (3 H, s); ^{13}C NMR ($CDCl_3$) δ 168.66 (s), 134.79 (d), 130.69 (s), 123.58 (d), 110.80 (d), 79.14 (d), 78.74 (s), 57.11 (q), 50.91 (q), 24.13 (q), 17.86 (q).

(15) Compound **16** was a solid: mp 101–103 °C; ^{1}H NMR ($CDCl_3$) δ 7.53 (1 H, br s, NH), 6.44 (1 H, br d, J = 4 Hz), 6.18–5.67 (2 H, m), 3.84 (1 H, d, J = 4 Hz), 3.39 (3 H, s), 3.27 (3 H, s), 2.05 (3 H, s), 1.34 (3 H, s); ^{13}C NMR ($CDCl_3$) δ 169.29 (s), 133.31 (d), 131.61 (s), 123.12 (d), 108.46 (d), 78.91 (d), 73.64 (s), 56.60 (q), 50.96 (q), 23.70 (q), 22.39 (q). A single-crystal X-ray analysis of **16** established it to have the designated stereochemistry with the vicinal methoxy groups cis. Details will be provided in a full paper on this subject.