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CHEMISTRY OF ACRONYCINE, XI. REARRANGEMENT OF DIHYDRONORACRONYCINE TO DIHYDROISONORACRONYCINE-MECHANISTIC STUDIES[1]

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ABSTRACT.—The rearrangement of dihydronoracronycine (**3**) to dihydroisonoracronycine (**4**) proceeds by way of an intermolecular reaction rather than an intramolecular reaction as had originally been supposed. Deuterium-labeling studies showed the incorporation of deuterium at C_3, C_{12}, and the geminal methyl positions of dihydroisonoracronycine (**4**). Because no reaction occurred when the bisnor derivative was treated in the same manner as **3**, it appears that the chromene geminal methyl groups are important for the rearrangement to occur.

Acronycine (**1**), a hemiterpene acridone alkaloid isolated from the bark of *Baurella simplicifolia* (Endl.) Hartley (Rutaceae) (2-8), the Australian scrub ash indigenous to New South Wales and Queensland, possesses the broadest spectrum of in vivo antineoplastic activity of any alkaloid thus far tested (9-12). However, relatively little is known of the chemistry or mode of action of **1** or its simple derivatives such as **2**.

We previously reported that if dihydronoracronycine (**3**) was dissolved in 98% H_2SO_4 and stirred under N_2 at room temperature for 24 h, rearrangement or removal of the prenyl moiety occurred, to afford dihydroisonoracronycine (**4**) and 1,3-dihydroxy-10-methyl acridone (**5**) (13). In addition to these two compounds, a compound with a uv spectrum similar to **4** and **5** was also isolated from the reaction mixture. In the ^{1}H-nmr spectrum, this compound displayed resonances similar to those of dihydronoracronycine (**3**) (14) and dihydroisonoracronycine (**4**) (13). Methylation with Me_2SO_4 and K_2CO_3 afforded, after preparative tlc, a compound with M^+ *m/z* 323, isomeric with **6** (12). Methylation with CH_2N_2 afforded the same compound; consequently, the OH group was not hydrogen bonded.

In the 360 MHz ^{1}H-nmr spectrum of the methyl ether, a geminal methyl signal (δ 1.48, 6H), a pair of methylene signals coupled to each other (δ 1.85 and 2.68, each 2H), methoxyl and N-CH_3 signals (δ 3.80 and 3.97, each 3H), a sharp singlet (δ 6.30, 1H), and four coupled aromatic signals were observed. These data are very similar to those of both dihydroacronycine (**6**) (14) and dihydroisoacronycine (**7**) (15). We therefore conducted nOe experiments in order to deduce the structure. When the signal at δ 3.97 (3H, s) was irradiated, a 19% nOe was observed at δ 6.30. On the other hand, when the signal at δ 6.30 was irradiated, 6% nOe effects were observed at δ 3.80 and 3.97.

From these data, the structure of this compound was determined to be **8** and the compound isolated from the reaction mixture to be **9**. Irradiation at δ 3.80 produced nOe effects at δ 6.30 and 7.39 of 18% and 16%, respectively. The resonance at δ 3.80 was therefore assigned to the N-CH_3 and the signal at δ 3.97 to the OCH_3. The same compound **8** was obtained previously by Rastogi *et al.* (16) through cyclization of **10** with HCOOH (98%) at 90° for 4 h.

When the reaction was conducted in H_2SO_4 for only 1 h, the isolate we obtained had a quite different character. The molecular weight of this compound was 377 instead of 309, suggesting the presence of two prenyl moieties on the acridone nucleus. The

[1]For the previous paper in this series see Funayama, *et al.* '(1).

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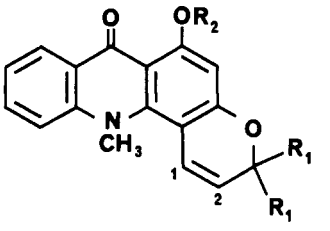
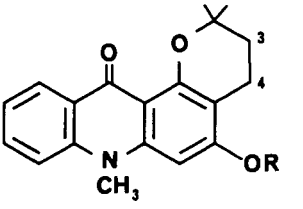
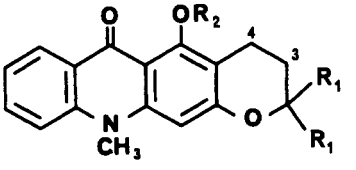


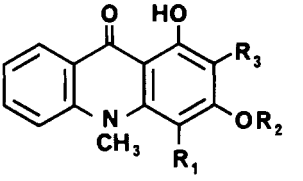
	R_1	R_2	
1	CH_3	CH_3	
2	CH_3	H	
3	CH_3	H	1,2-H_2
6	CH_3	CH_3	1,2-H_2
14	H	H	
16	H	H	1,2-H_2



	R	
8	CH_3	
9	H	
18	CH_3	Δ-3,4



	R_1	R_2	
4	CH_3	H	
7	CH_3	CH_3	
13	H	H	Δ-3,4
15	H	H	
17	CH_3	CH_3	Δ-3,4

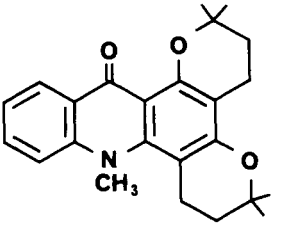


	R_1	R_2	R_3
5	H	H	H
10	H	CH_3	$-CH_2CH=C(CH_3)_2$
19	$-CH_2CH=C(CH_3)_2$	H	H
20	$-CH_2CH=C(CH_3)_2$	CH_3	H
21	H	CH_3	H

^{1}H-nmr spectrum also exhibited the presence of two prenyl moieties, namely, two sets of geminal methyl signals (δ 1.44 and 1.45, each 6H), four methylene signals (δ 1.75, 1.82, 2.63, and 2.86, each 2H), one N-CH_3 (δ 3.74, 3H), and four coupled aromatic signals. Structure **11** was deduced from these accumulated data, and this strongly suggested the occurrence of an *inter*molecular reaction during the rearrangement reaction of **3** to **4**.

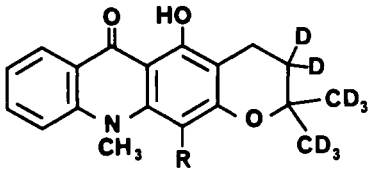
In order to further investigate the mechanism of the facile conversion of **3** to **4**, the same reaction was conducted using 98% D_2SO_4 instead of 98% H_2SO_4. Thus, dihydronoracronycine (**3**) was dissolved in 98% D_2SO_4, and this solution was stirred under N_2 for 24 h. From the reaction mixture, the compound corresponding to **4** was purified.

In its ^{1}H-nmr spectrum, signals for a N-CH_3 and a hydrogen-bonded phenolic proton, in addition to four coupled aromatic protons, were observed. However, only one



11

methylene signal, assigned to the 4-CH_2, was apparent, and the singlet aromatic resonance was also reduced in intensity. Quite unexpectedly, the geminal methyl signals had disappeared from the spectrum. Mass spectral analysis indicated this compound to have M^+ *m/z* 317 instead of *m/z* 309 expected for proto-dihydroisonoracronycine (**4**). Analysis of these data indicated that eight to nine deuteriums had been incorporated into the dihydronoracronycine (**3**) molecule during the rearrangement reaction, and that deutero-dihydroisonoracronycine had the structure **12**. These results suggest that a protonation-deprotonation mechanism involving the prenyl moiety occurs during the rearrangement reaction.



12 R=D~20%
R=H~80%

To establish the need for the geminal methyl signals in the rearrangement, the chemistry of the corresponding bis-nor derivative was studied. When a 1:6 mixture of **13** and **14** (17) was catalytically hydrogenated, two dihydro derivatives, **15** and **16**, were isolated. The skeletons of these compounds were confirmed by nOe experiments. Namely, when δ 3.78 (N-CH_3) of **15** was irradiated, nOe effects were observed at δ 6.30 (23%) and 7.48 (14%). On the other hand, irradiation at δ 3.87 (N-CH_3) of **16** caused nOe effects at δ 2.92 (7%) and 7.43 (18%).

The angular compound **16** was dissolved in 98% H_2SO_4, and after 24 h, the reaction mixture was processed in the usual manner. Only the starting material, **16**, was detected by tlc, and none of the linear compound **15**. It was therefore concluded that the geminal methyl moiety of the acronycine skeleton is necessary for rearrangement to the isoacronycine skeleton.[3]

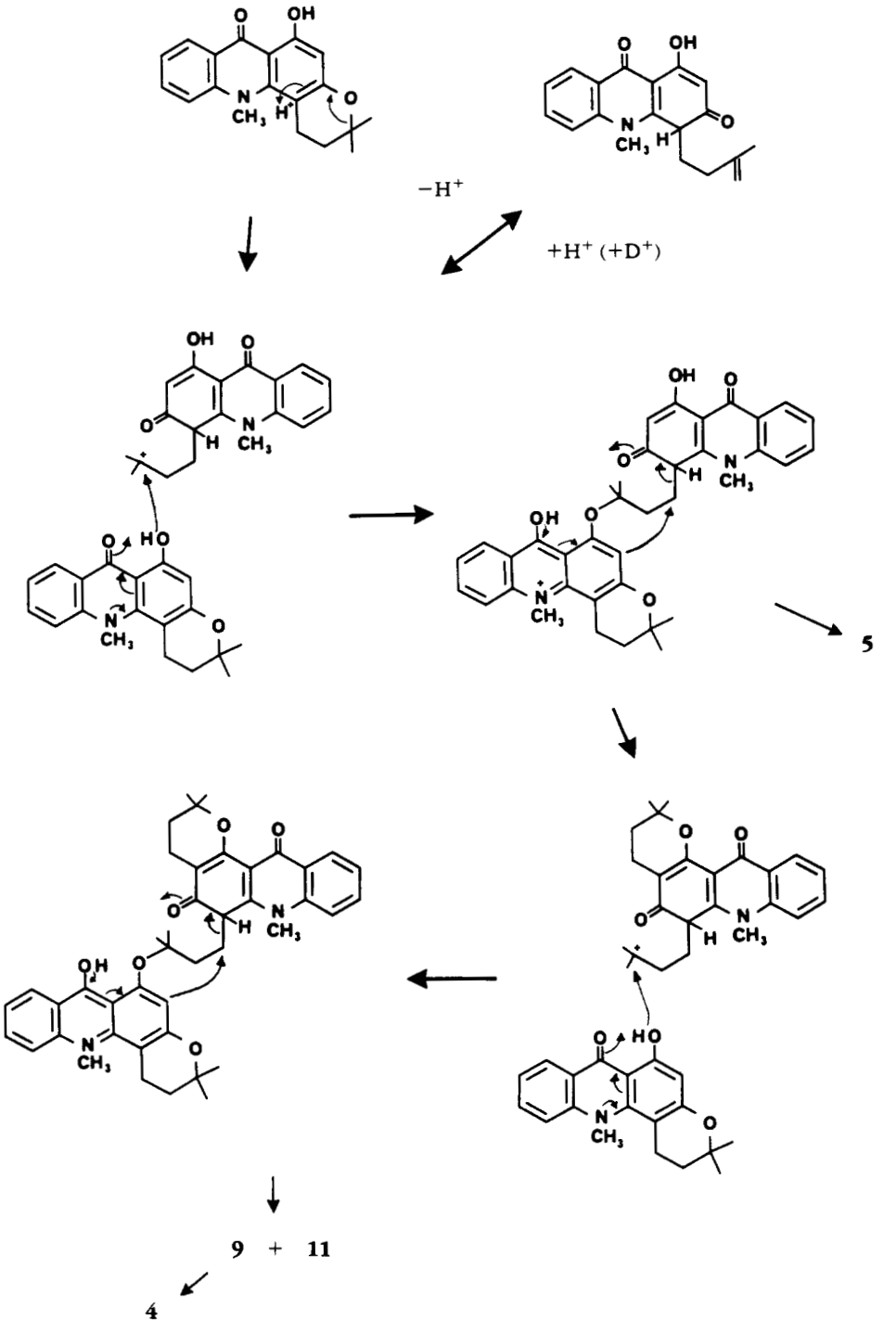
Through these experiments there was established the possibility of an intermolecular rearrangement in the transformation of dihydronoracronycine (**3**) to dihydroisonoracronycine (**4**), which leads to disproportionation and the formation of dihydroisonoracronycine (**3**), 1,3-dihydroxy-10-methyl acridone (**5**), **9**, and **11** (Scheme 1). The complex array of products formed through the *inter*molecular reactions of noracronycine (**2**) have been described previously (1, 18-21).

It is interesting that acronycine (**1**), noracronycine (**2**), and dihydronoracronycine (**3**) exhibit quite distinct reactions with 98% H_2SO_4. When **1** was treated with 98% H_2SO_4, no reaction was observed (21). On the other hand, a dimer was obtained by treating noracronycine (**2**) with 98% H_2SO_4 (21), and dihydroisonoracronycine (**4**), 1,3-dihydroxy-10-methyl acridone (**5**), **9**, and **11** were obtained by treating dihydronoracronycine (**3**) with 98% H_2SO_4.

We have previously reported that acronycine (**1**) could be converted into isoacronycine (**17**) (13), but not the reverse. From the results obtained herein, it became evident that acronycine (**1**) could be further converted into **8**, or theoretically even **18**, by treating **8** with DDQ. Studies along these lines will be reported subsequently.

During the course of this work, Wu *et al.* (22) reported that treatment of glycocitrine-II (**19**) with HCOOH (85%) at 90° for 4 h afforded **3**, **4**, and **11**. On the other hand, when 3-*O*-methyl-glycocitrine-II (**20**) was treated similarly, **8** and **21** were ob-

[3]A reviewer has correctly pointed out that a monomethyl substituted derivative may also undergo rearrangement.



tained. From these results, the existence of intermolecular reactions during these cyclization reactions was postulated.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.— Melting points were determined using a Kofler hot-stage microscope and are uncorrected. Uv spectra were recorded on a Beckman model DB-G spec-

trophotometer and ir spectra on a Nicolet model MX-1 FT-IR interferometer. ^{1}H-nmr spectra were recorded on a Nicolet NT-360 instrument at the NSF Regional NMR Facility at the University of Illinois at Urbana, Urbana-Champaign, or with a Varian T-60A instrument operating at 60 MHz with a Nicolet Model TT-7 Fourier Transform attachment. Silica gel GHLF (Analtech) was used for preparative tlc.

ACRONYCINE (**1**), NORACRONYCINE (**2**) AND DIHYDRONORACRONYCINE (**3**).—The preparation and physical and spectral properties of these compounds were described previously (14,23).

TREATMENT OF DIHYDRONORACRONYCINE (**3**) WITH 98% H_2SO_4 AT ROOM TEMPERATURE FOR 24 H.—The reaction procedures and chemical and spectral properties of dihydroisonoracronycine (**4**) and 1,3-dihydroxy-10-methyl acridone (**5**) were described previously (16). The third compound **9** was isolated as a pale yellow powder by concentrating the $CHCl_3$ layer [yield: 270 mg from 1.02 g of dihydronoracronycine (**3**)]; uv (EtOH) λ max 248, 263, 272, 304, 328, and 387 nm; ^{1}H nmr (360 MHz, acetone-d_6) δ 1.40 (6H, s, gem. CH_3), 1.82 (2H, t, J=6.9 Hz, 3-CH_2), 2.66 (2H, t, J=6.8 Hz, 4-CH_2), 3.77 (3H, s, N-CH_3), 6.61 (1H, s, C_6-H), 7.20 (1H, t, J=7.4 Hz, C_{10}-H), 7.59 (1H, d, J=8.5 Hz, C_8-H), 7.66 (1H, dt, J=1.4, 7.0 Hz, C_9-H), 8.32 (1H, d, J=7.9 Hz, C_{11}-H), and 9.50 (1H, s, D_2O exchangeable, C_5-OH).

METHYLATION OF **9** WITH Me_2SO_4/K_2CO_3IN Me_2CO.—The pale yellow powder of **9** (21.8 mg) was suspended in Me_2CO (10 ml), and to this suspension Me_2SO_4 (0.3 ml) and K_2CO_3 (anhydrous, 400 mg) were added and refluxed on a steam bath for 4 h. After adding fresh Me_2SO_4 (0.3 ml) and K_2CO_3 (400 mg), the reaction was continued for 16 h.

The reaction mixture was poured into H_2O and stirred for 15 min and extracted with $CHCl_3$ (2×100 ml). The $CHCl_3$ layer was treated with 5% NH_4OH (100 ml), washed with H_2O (100 ml), dried over Na_2SO_4 (anhydrous), and the filtrate concentrated in vacuo to afford a pale yellow powder that was purified by preparative tlc to yield **8** (5.1 mg) as a yellow powder; ir (KBr) ν max 3486, 3436, 1611, 1594, 1561, 1501, 1479, 1234, 1159, 1120, 1107, and 748 cm^{-1}; uv ($CHCl_3$) λ max 252 (sh), 266 (sh), 276, 306 (sh), 372 (sh), and 388 nm; ms *m/z* 324 (17%), 323 (M^+, 75), 308 (7), 280 (13), 269 (18), 268 (100), 238 (10), 225 (6), and 210 (4); ^{1}H nmr (360 MHz, $CDCl_3$) δ 1.48 (6H, s, gem. CH_3), 1.85 (2H, t, J=6.8 Hz, 3-CH_2), 2.68 (2H, t, J=6.9 Hz, 4-CH_2), 3.80 (3H, s, N-CH_3), 3.97 (3H, s, O-CH_3), 6.30 (1H, s, C_6-H), 7.21 (1H, t, J=7.4 Hz, C_{10}-H), 7.39 (1H, d, J=8.5 Hz, C_8-H), 7.60 (1H, t, J=7.9 Hz, C_9-H), and 8.53 (1H, d, J=8.0 Hz, C_{11}-H).

METHYLATION OF **9** WITH CH_2N_2.—The pale yellow powder of **9** (5.0 mg) was suspended in 0.5 ml of EtOH. To this suspension, CH_2N_2 in Et_2O (1 ml) was added and the mixture stirred for 16 h. Through preparative tlc, **8** (3.6 mg) was obtained as a pale yellow powder.

TREATMENT OF DIHYDRONORACRONYCINE (**3**) WITH 98% H_2SO_4FOR 1 H AT ROOM TEMPERATURE.—Dihydronoracronycine (**3**, 20.4 mg) was dissolved in 98% H_2SO_4 (5.0 ml) and the mixture stirred under N_2 at room temperature. After 1 h, the solution was diluted with H_2O (50 ml) and extracted with $CHCl_3$ (2×50 ml). The combined $CHCl_3$ layers were washed with H_2O, dried over anhydrous Na_2SO_4, and concentrated in vacuo to afford a yellow powder (16.0 mg). By preparative tlc of this fraction, **11** was obtained as a pale yellow powder (2.9 mg); ir (KBr) ν max 2917, 2849, 1588, 1493, 1462, 1453, 1325, 1276, 1156, and 749 cm^{-1}; uv ($CHCl_3$) λ max 252 (sh), 277, 304, 322, and 388 nm; ms *m/z* 377 (M^+, 27), 322 (36), 306 (10), 278 (16), 267 (18), 266 (100), 238 (12), 237 (11), 212 (16), 210 (11), 208 (12), 184 (7), 180 (11), 167 (11), 166 (8), 115 (8.5), and 77 (23); ^{1}H nmr (360 MHz, $CDCl_3$) 1.445 (6H, s, gem CH_3), 1.454 (6H, s, gem CH_3), 1.75 (2H, t, J=6.5 Hz, 2-CH_2 or 6-CH_2), 1.82 (2H, t, J=6.9 Hz, 6-CH_2 or 2-CH_2), 2.63 (2H, t, J=6.8 Hz, 1-CH_2 or 5-CH_2), 2.86 (2H, t, J=6.4 Hz, 5-CH_2 or 1-CH_2), 3.74 (3H, s, N-CH_3), 7.17 (1H, t, J=7.7 Hz, C_{11}-H), 7.31 (1H, d, J=8.4 Hz, C_{13}-H), 7.56 (1H, dt, J=1.0, 8.0 Hz, C_{12}-H), and 8.34 (1H, dd, J=1.1, 7.6 Hz, C_{10}-H).

TREATMENT OF DIHYDRONORARONYCINE (**3**) WITH D_2SO_4 AT ROOM TEMPERATURE.—Dihydronoracronycine (**3**, 17.2 mg) was dissolved in 98% D_2SO_4 (5.0 ml) and the solution stirred for 24 h under a N_2 atmosphere. The reaction mixture was poured into cold H_2O (50 ml) and extracted with $CHCl_3$ (2×100 ml). The combinerd $CHCl_3$ layers were washed with 5% $NaHCO_3$ solution and H_2O (each 50 ml), dried over anhydrous Na_2SO_4, and concentrated in vacuo to afford a brownish yellow powder (8.4 mg). Through preparative tlc eluting with $CHCl_3$ as the solvent, deutero-dihydroisonoracronycine (**12**) was isolated as a yellow powder; ms *m/z* 319 (8%), 318 (35), 317 (84), 316 (19), 315 (8), 314 (8), 299 (9), 268 (22), 267 (13), 257 (14), 256 (45), 255 (100), 254 (39), 253 (17), 243 (17), 226 (17), 225 (42), 182 (11), 141 (10), and 140.5 (11); ^{1}H nmr (60 MHz, $CDCl_3$) δ 2.74 (bs, 2H, 4-CH_2), 3.71 (3H, s, N-CH_3), 6.25 (0.2 H, s, C_{12}-H), 7.24 (1H, dt, J=1, 7 Hz, C_8-H), 7.40 (1H, dd, J=1.7 Hz, C_{10}-H), 7.55 (1H, dt, J=1.7 Hz, C_9-H), 8.44 (1H, dd, J=2, 8 Hz, C_7-H), and 15.08 (1H, s, 5-OH).

HYDROGENATION OF 5-HYDROXY-11-METHYL-2*H*-PYRANO[3,2-B]ACRIDIN-6(11-*H*)-ONE (**13**)

AND 6-HYDROXY-12-METHYL-3*H*-PYRANO[2,3-C]ACRIDIN-7(12*H*)-ONE (**14**).—A mixture of **13** and **14** (1:6, 5.1 mg) was dissolved in EtOAc (5.0 ml). To this solution, Pd-C (5%, 1.0 mg) and a drop of HOAc were added and H_2 gas was introduced and the mixture stirred overnight. The reaction mixture was filtered and two compounds were isolated by preparative tlc. Linear derivative **15** (0.8 mg) was obtained as a pale yellow powder; ms *m/z* 282 (19%), 281 (M^+, 100), 280 (38), 266 (38), 254 (10), 253 (37), 252 (12), 238 (10), 226 (10), 225 (43), and 182 (13); ^{1}H nmr (360 MHz, $CDCl_3$) δ 2.06 (2H, m, 3-CH_2), 2.78 (2H, t, J=6.5 Hz, 4-CH_2), 3.78 (3H, s, N-CH_3), 4.28 (2H, t, J=5.3 Hz, 2-CH_2), 6.30 (1H, s, C_{12}-H), 7.33 (1H, t, J=7.1 Hz, C_8-H), 7.48 (1H, d, J=8.6 Hz, C_{10}-H), 7.72 (1H, t, J=7.9 Hz, C_9-H), 8.48 (1H, d, J=7.7 Hz, C_7-H), and 15.13 (1H, s, C_5-OH).

The angular derivative **16** (3.8 mg) was obtained as yellow powder; uv ($CHCl_3$) λ max 253, 267, 278, 303, 342 (sh), and 400 nm; ms *m/z* 282 (19%), 281 (M^+ 100), 280 (16), 266 (8), 254 (9), 253 (57), 226 (9), 225 (52), 196 (8), 182 (6), 168 (5), 154 (5), and 140.5 (5); ^{1}H nmr (360 MHz, $CDCl_3$) δ 1.95 (2H, m, 2-CH_2), 2.92 (2H, t, J=6.1 Hz, 1-CH_2), 3.87 (3H, s, N-CH_3), 4.32 (2H, t, J=5.3 Hz, 3-CH_2), 6.25 (1H, s, C_5-H), 7.30 (1H, t, J=7.5 Hz, C_9-H), 7.43 (1H, d, J=8.5 Hz, C_{11}-H), 7.71 (1H, t, J=7.8 Hz, C_{10}-H), 8.36 (1H, d, J=7.8 Hz, C_8-H), and 14.31 (1H, s, C_6-OH).

TREATMENT OF **16** WITH 98% H_2SO_4 AT ROOM TEMPERATURE.—The angular derivative **16** (2.2 mg) was dissolved in 98% H_2SO_4 (2.2 ml) and the solution stirred under N_2 at room temperature. After 24 h, the solution was diluted with H_2O (20 ml) and extracted with $CHCl_3$ (2×20 ml). The combined $CHCl_3$ layers were washed with 5% $NaHCO_3$ solution and H_2O (each 20 ml) and dried over anhydrous Na_2SO_4 and concentrated in vacuo to yield the starting material **16** (1.8 mg). None of the linear isomer **15** was detected by tlc.

ACKNOWLEDGMENTS

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