dinates, thermal parameters, and bond angles and lengths for **1** and **2** (8 pages); tables of calculated and observed structure factors (29 pages). Ordering information is given on any current masthead page.

Total Synthesis of Ptaquilosin: The Aglycon of Ptaquiloside, a Potent Bracken Carcinogen

Hideo Kigoshi, Yoshifumi Imamura, Haruki Niwa, and Kiyoyuki Yamada*

Department of Chemistry, Faculty of Science Nagoya University, Chikusa, Nagoya 464, Japan

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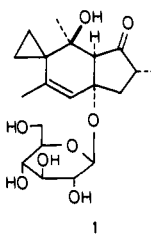
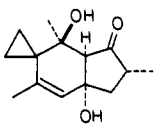
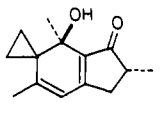
Since the carcinogenicity of bracken fern (*Pteridium aquilinum*) was discovered in 1960,[1] isolation of the carcinogen(s) has been a long-standing problem. We isolated a new type of carcinogen ptaquiloside (**1**) from bracken in 1983, determined the novel structure,[2] and proved its potent carcinogenicity.[3] Both ptaquiloside (**1**) and its aglycon ptaquilosin (**2**) are converted under weakly basic or neutral conditions into dienone **3**,[2a,d] which is the active form of **1** and causes base-specific cleavage of DNA.[4] The first total synthesis of optically active ptaquilosin (**20**), the enantiomer of natural **2** is described herein.



1



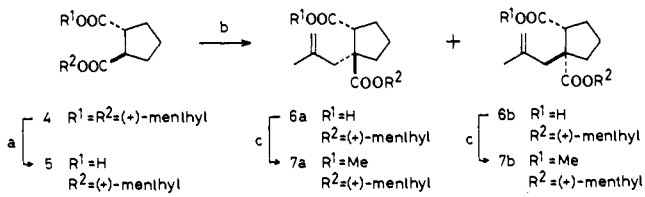
2



3

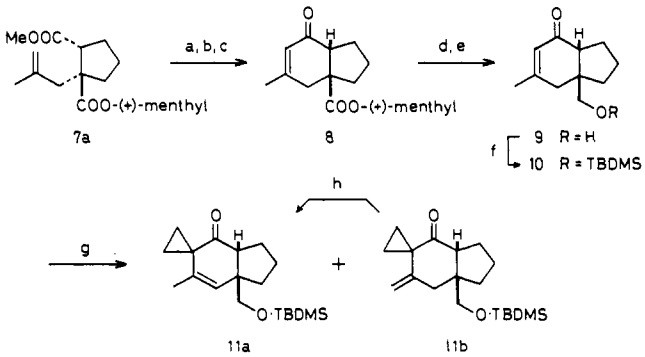
(+)-Dimenthyl (1*R*,2*R*)-cyclopentane-1,2-dicarboxylate (**4**) prepared according to the Yamamoto method[5] was partially hydrolyzed to give monomenthyl ester **5**.[6] The dianion generated from **5** (2.4 equiv of LDA, THF) reacted with methallyl chloride to afford a 4:1 mixture of diastereomeric esters, **6a** and **6b** (86%), which, after conversion into the corresponding methyl esters, was separated by chromatography on silica gel to give **7a** (77%) and **7b** (19%) (Scheme I). Contrary to the expectation the major diastereomer has the stereostructure **6a**.[7,8] The methyl ester group

Scheme I[a]



[a] (a) KOH, 30% H_2O_2, MeOH, 50 °C, 14 h; (b) LDA (2.4 equiv), THF, −25 °C, 1 h, then CH_2=C(Me)CH_2Cl (3.2 equiv), 23 °C, 16 h; (c) CH_2N_2, ether, 23 °C, 5 min.

Scheme II[a]



[a] (a) KOH, *i*-PrOH/H_2O (10:1), reflux, 6 h; (b) $(COCl)_2$, benzene, 23 °C, 3 h; (c) $SnCl_4$, CH_2Cl_2, −78 °C, 2 h; (d) $LiAlH_4$, THF, 23 °C, 50 min; (e) imidazolium dichromate, DMF, 23 °C, 1.5 h; (f) *t*-$BuMe_2SiCl$, imidazole, DMF, 23 °C, 45 min; (g) $ClCH_2CH_2SMe_2$·I, KI, *t*-BuOK, *t*-BuOH, 23 °C, 2 h; (h) *p*-TsOH, dioxane, reflux, 1 h.

in **7a** was transformed via a two-step process into the acid chloride, which was subjected to cyclization with Lewis acid to give bicyclic enone **8** (81% from **7a**) (Scheme II). Conversion of **8** into enone **9** (81%) was accomplished by the following sequence: (1) reduction with $LiAlH_4$ and (2) oxidation with imidazolium dichromate.[9] A single recrystallization of this material (pentane/ether) provided pure **9**, mp 45–47 °C (>99% ee),[10] and subsequently silylation of **9** furnished enone **10** (quantitative). Spirocyclopropanation of **10** was effected by using 2-chloroethyldimethylsulfonium iodide[11] to form a separable 3:1 mixture of two ketones, **11a** (42%) and **11b** (15%), the latter **11b** being isomerized by acid catalysis[12] to the former **11a** (95%). Conversion of **11a** to conjugated ketone **12** (82%) was performed in two straightforward steps (Scheme III). Oxidation of the double bond conjugated with the keto group in **12** afforded epoxide **13**[13a] (88%), which on reduction (Ca, liquid NH_3/THF, −78 °C) provided β-hydroxy ketone **14** (91%). The reaction of the Grignard reagent (MeMgI) with **14** proceeded highly stereoselectively from the less hindered, convex face of the substrate and gave diol **15a**[13b] (89%),

(1) (a) Evans, I. A. In *Chemical Carcinogens*, 2nd ed.; Searle, C. E., Ed.; American Chemical Society: Washington, DC, 1984; Vol. 2, pp 1171–1204. (b) Hirono, I.; Yamada, K. In *Naturally Occurring Carcinogens of Plant Origin*; Hirono, I., Ed.; Kodansha-Elsevier: Tokyo, Amsterdam, 1987; pp 87–120.

(2) (a) Niwa, H.; Ojika, M.; Wakamatsu, K.; Yamada, K.; Hirono, I.; Matsushita, K. *Tetrahedron Lett.* **1983**, *24*, 4117. (b) Niwa, H.; Ojika, M.; Wakamatsu, K.; Yamada, K.; Ohba, S.; Saito, Y.; Hirono, I.; Matsushita, K. *Tetrahedron Lett.* **1983**, *24*, 5371. (c) Ohba, S.; Saito, Y.; Hirono, I.; Niwa, H.; Ojika, M.; Wakamatsu, K.; Yamada, K. *Acta Crystallogr., Sect. C*, **1984**, *40*, 1877. (d) Ojika, M.; Wakamatsu, K.; Niwa, H.; Yamada, K. *Tetrahedron* **1987**, *43*, 5261.

(3) (a) Hirono, I.; Yamada, K.; Niwa, H.; Shizuri, Y.; Ojika, M.; Hosaka, S.; Yamaji, T.; Wakamatsu, K.; Kigoshi, H.; Niiyama, K.; Uosaki, Y. *Cancer Lett.* **1984**, *21*, 239. (b) Hirono, I.; Aiso, S.; Yamaji, T.; Mori, H.; Yamada, K.; Niwa, H.; Ojika, M.; Wakamatsu, K.; Kigoshi, H.; Niiyama, K.; Uosaki, Y. *Gann* **1984**, *75*, 833. (c) Hirono, I.; Ogino, H.; Fujimoto, M.; Yamada, K.; Yoshida, Y.; Ikagawa, M.; Okumura, M. *J. Natl. Cancer Inst.* **1987**, *79*, 1143.

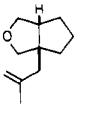
(4) Ojika, M.; Sugimoto, K.; Nozaki, N.; Okazaki, T.; Yamada, K., unpublished results.

(5) Misumi, A.; Iwanaga, K.; Furuta, K.; Yamamoto, H. *J. Am. Chem. Soc.* **1985**, *107*, 3343.

(6) All new compounds exhibited satisfactory spectral (IR, ^{1}H NMR, MS) and exact mass spectral data.

(7) For contrasteric alkylation, see: (1) Naef, R.; Seebach, D. *Angew. Chem., Int. Ed. Engl.* **1981**, *20*, 1030. (2) Ladner, W. *Angew. Chem., Int. Ed. Engl.* **1982**, *21*, 449. (3) Ladner, W. *Chem. Ber.* **1983**, *116*, 3413.

(8) Stereochemistry of **6a** and **6b** was determined as follows: **7b** could be converted into a tetrahydrofuran derivative i in two steps (1. $LiAlH_4$; 2. TsCl-pyr), whereas **7a** could not.



i

(9) Kim, S.; Lhim, D. C. *Bull. Chem. Soc. Jpn.* **1986**, *59*, 3297.

(10) The enantiomeric purity of this compound was determined by analyzing the ^{1}H NMR spectrum of derived **10** in the presence of chiral shift reagent Eu(hfc)$_3$.

(11) For spirocyclopropanation of saturated ketones with this reagent, see: Ruder, S. M.; Ronald, R. C. *Tetrahedron Lett.* **1984**, *25*, 5501.

(12) Cf. Yates, P.; Helferty, P. H.; Mahler, P. *Can. J. Chem.* **1983**, *61*, 78.

(13) A small amount of the diastereomer was also obtained: (a) 7%; (b) 3%.

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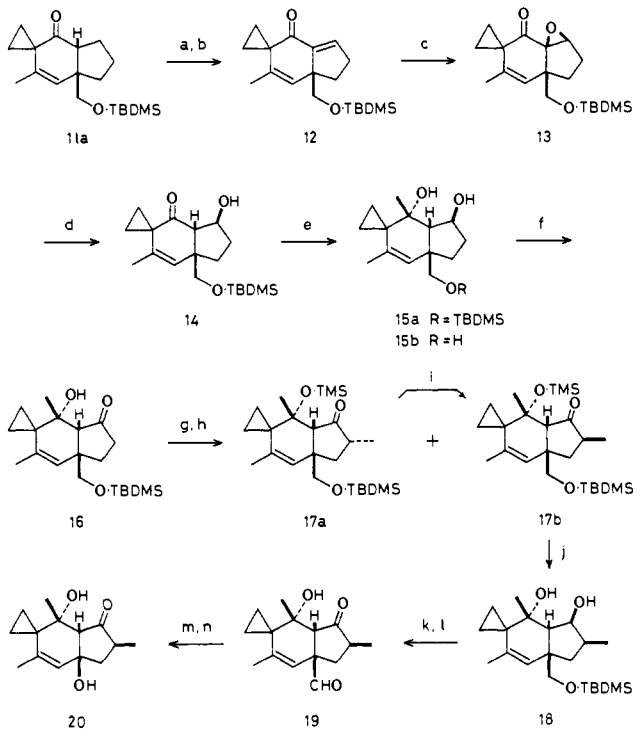
Scheme III[a]



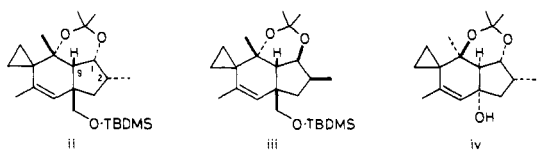
[a](a) LDA, then PhSeCl, THF, −78 °C, 1 h; (b) 30% H_2O_2, pyr, CH_2Cl_2, 23 °C, 40 min; (c) 30% H_2O_2, NaOH, MeOH, 10 °C, 6.5 h; (d) Ca, liquid NH_3/THF (2:1), −78 °C, 2 h; (e) MeMgI, ether, 23 °C, 1.5 h; (f) DMSO, $(COCl)_2$, CH_2Cl_2, −60 °C, 15 min, then Et_3N, −60 °C → 23 °C, 30 min; (g) LDA (10 equiv), then Me_3SiCl, DME, 0 °C, 15 min → 23 °C, 1 h; (h) $PhCH_2NMe_3{\cdot}F$, MeI, molecular sieves 4A, THF, 23 °C, 2 h; (i) *t*-BuOK, *t*-BuOH, 30 °C, 4 h; (j) $LiAlH_4$, ether, 23 °C, 30 min; (k) Bu_4NF, THF, 45 °C, 23 h; (l) DMSO, $(COCl)_2$, CH_2Cl_2, −68 °C, 15 min, then Et_3N, −68 °C → 23 °C, 35 min; (m) O_2, EtOAc, 50 °C, 18 h; (n) PPh_3, ether, 23 °C, 1 h.

Swern oxidation of which furnished ketone **16** (98%). The X-ray crystallographic analysis of racemic triol **15b**[14] obtained by desilylation of racemic **15a**[14] (Bu_4NF, THF) confirmed the assigned stereochemistry of **15a** as indicated. Monomethylation α to the keto group in **16** was executed by the Kuwajima procedure:[15] the enol silyl ether prepared from **16** reacted with MeI in the presence of $PhCH_2NMe_3F$ to give a separable mixture of two diastereomers, **17a** (37%) and **17b** (14%), the former **17a** being converted into the latter **17b** by base treatment (75%). The thermodynamically more stable isomer **17b** was shown to have the desired stereochemistry regarding the secondary methyl group.[16] Transformation of **17b** into aldehyde **19** (95% overall) was effected through the sequence: (1) reduction of the keto group and removal of the TMS group to give **18**; (2) deprotection of the TBDMS group; (3) Swern oxidation.

(14) Mp of racemic **15b**, 146–148 °C. Racemic **15a** was available by an alternative synthetic route starting from α-allyl-δ-valerolactone: Kigoshi, H.; Sawada, A.; Nakayama, Y.; Niwa, H.; Yamada, K., unpublished results.

(15) (a) Kuwajima, I.; Nakamura, E. *J. Am. Chem. Soc.* **1975**, *97*, 3257. (b) Kuwajima, I.; Nakamura, E.; Shimizu, M. *J. Am. Chem. Soc.* **1982**, *104*, 1025.

(16) Stereochemistry of the secondary methyl groups in **17a** and **17b** was established by the ^1H NMR spectral analysis: **17a** and **17b** were converted in two steps (1. $LiAlH_4$; 2. $CH_2{=}C(Me)OMe$, H^+) into conformationally rigid derivatives, ii and iii, respectively, and their coupling constants (ii, $J_{1,2} = J_{1,9} = 4.0$ Hz; iii, $J_{1,2} = J_{1,9} = 9.6$ Hz) were compared with those ($J_{1,2} = J_{1,9} = 9.7$ Hz) of the compound iv derived from natural **1**.



The final phase of the synthesis was oxidative removal of the angular formyl group in **19** to introduce a hydroxyl group at the ring juncture under the conditions mild enough for the unstable product ptaquilosin (**20**) to survive. Thus, the concentrated solution of **19** in EtOAc under the oxygen atmosphere was warmed at 50 °C to afford a hydroperoxide,[17] which was reduced with PPh_3 providing (+)-ptaquilosin (**20**)[18] (37%) as a colorless oil, identical with natural (−)-**2**[18,19] in every respect (^1H NMR, IR, MS, α_D, TLC) except for the sign of specific rotation.

Acknowledgment. We thank Prof. H. Yamamoto and Dr. K. Furuta, Faculty of Engineering, Nagoya University for providing us with the experimental details for the preparation of **4** prior to publication. This work was supported in part by Grant-in-Aids for Encouragement of Young Scientists (No. 60740290 and 62740308 to H.K.) from the Ministry of Education, Science, and Culture, Japan.

Supplementary Material Available: Spectral and physical data for compounds **5** and **7–20** and X-ray crystallographic data for racemic **15b** (12 pages). Ordering information is given on any current masthead page.

(17) This deformylation–oxygenation reaction could also be effected in benzene at 50 °C in the presence of AIBN in less yield.

(18) Synthetic **20**: $[\alpha]_D^{20}$ +232° (*c* 0.17, $CHCl_3$). Natural **2**: $[\alpha]_D^{20}$ −246° (*c* 0.82, $CHCl_3$).

(19) Natural **2** was derived from **1** by chemical means: Kigoshi, H.; Sawada, A.; Imamura, Y.; Niwa, H.; Yamada, K., unpublished results.

DNA Structural Data from a Dynamics Probe. The Dynamic Signatures of Single-Stranded, Hairpin-Looped, and Duplex Forms of DNA Are Distinguishable

Andreas Spaltenstein, Bruce H. Robinson, and Paul B. Hopkins*

Department of Chemistry, University of Washington Seattle, Washington 98195

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Efforts to establish structure-function relationships involving nucleic acids have focused attention upon a variety of non-B conformations of DNA, for example, A-,Z-bent, and hairpin-looped conformations.[1] When such features are embedded within B-DNA, as would be the case in vivo, spectroscopic structural assignment is complicated because the region of interest constitutes only a small portion of the macromolecule. The presence of unusual structures within large DNA's is often inferred from differential chemical reactivity;[2] the possibility of dynamic equilibrium among two or more DNA conformations complicates interpretation of such data. Spectroscopic methods which provide information about structural elements which constitute a small portion of the DNA are thus of great interest. EPR spectroscopy has been widely used to monitor local dynamic features of macromolecules; should a correlation of DNA local structure and dynamics exist, the EPR technique in combination with site-specific DNA spin labeling[3] would become a powerful tool in DNA structural studies.

We have previously reported that a nitroxide spin-labeled analogue of thymidine (e.g., **1**, T*) may be incorporated by automated chemical synthesis into deoxyoligonucleotides and that this probe does not significantly perturb the solution B-structure of the duplex form of 5′-d(CGCGAATT*CGCG).[3] EPR studies of this duplex indicated that the spin probe's effective rotational

(1) (a) Weintraub, H. *Cell* **1983**, *32*, 1191. (b) Elgin, S. C. R. *Nature* **1984**, *309*, 213.

(2) Furlong, J. C.; Lilley, D. M. J. *Nucleic Acids Res.* **1986**, *14*, 3995 and references 18–25 cited therein.

(3) Spaltenstein, A.; Robinson, B. H.; Hopkins, P. B. *J. Am. Chem. Soc.* **1988**, *110*, 1299.

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