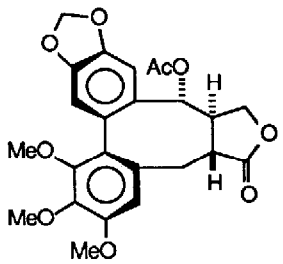
Tetrahedron Letters,Vol.30,No.22,pp 2949-2952,1989 0040-4039/89 $3.00 + .00
Printed in Great Britain Pergamon Press plc

DESIGN, SYNTHESIS, AND ANTITUMOR ACTIVITY OF STEGANACIN AZA-ANALOGUES

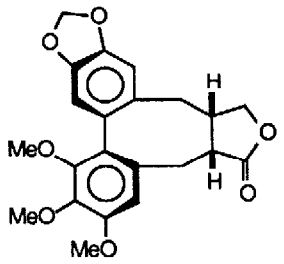
Kiyoshi Tomioka,* Yoshihiro Kubota, Hisashi Kawasaki, and Kenji Koga*
Faculty of Pharmaceutical Sciences, University of Tokyo,
Hongo, Bunkyo-ku, Tokyo 113, Japan

Summary: Artificial steganacin aza-analogue 3 was designed and synthesized in a quite efficient way. Aza-analogue 3 was shown to be endowed with promising in vitro and in vivo activity higher than natural steganacin (-)-1.

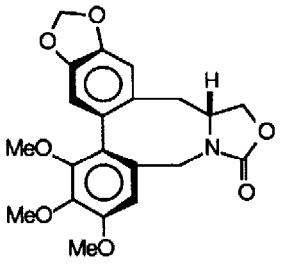
Power of organic synthesis is expected to play an important role in the creation of artificial compounds of promising biological activity.[1] In our program aimed at the creation of new compounds of antitumor activity based on the steganine lignans (for example, steganacin (-)-**1**[2~4]), we have shown that isopicrostegane ((-)-**2**),[5] one of the four skeleton isomers, shows a potent cytotoxicity higher than natural steganacin.[6] The mechanism of action of **2** was shown to be based on the inhibition of microtuble assembly.[7] Expecting to develop a new leading structure for anticancer drugs we started a next stage study. In the present communication we describe design, synthesis, and biological activity of new artificial isopicrostegane aza-analogue **3**. Furthermore, a boat-chair conformation of dibenzocyclooctadiene is proposed to be a biologically active form.



(-)-**1**



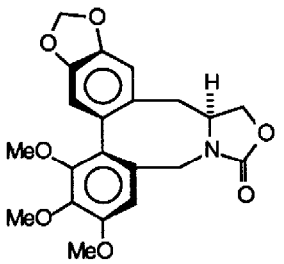
(-)-**2**



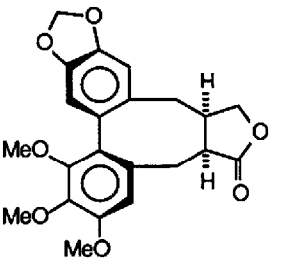
3

The guide lines we set for the creation of candidate was categorized as follows: (1) the stereochemical structure has the most similarity to isopicrostegane; (2) carbonyl oxygen should have enough electron density to form hydrogen-bond; (3) the compounds have minimum stereoisomers; (4) the synthetic route is as short as possible; (5) optically pure compounds are readily available.

On the basis of the guide lines we designed **3** as an aza-analogue of isopicrostegane (-)-**2**.[5] Atropisomerism and carbon center at the β-position of lactone carbonyl group of **3** have the same relative configuration and the center α to the carbonyl is a sp^2 nitrogen, reducing one asymmetric center. The isomeric compounds **4** is, however, analogue of picrostegane (-)-**5** which showed weak cytotoxicity.[6]



4



(-)-**5**

The synthesis began with the preparation of racemic amino acid **6**. According to the reported procedure[8] acetylaminomalonate was alkylated with piperonyl chloride to afford, after hydrolysis and decarboxylation, the corresponding known amino acid **6**. Reduction of **6** with lithium aluminum hydride and treatment of the corresponding amino alcohol **7** (mp 80-82 °C) with diethyl carbonate provided the urethane **8** (mp 82-83.5 °C).[9] Alkylation of **8** with trimethoxybenzyl bromide furnished **9** (mp 95.5-97.0 °C). Nonphenolic oxidative intramolecular coupling of **9** by the action of VOF_3 in methylene chloride-trifluoroacetic acid gave rise to a mixture of separable two diastereoisomers **3** (mp 185-186 °C) and **4** (mp 167-168 °C) in a ratio of 60:1 (determined by HPLC of crude products) in 98% combined yield.[3,10] The major product was assigned to **3** on the basis of ^{1}H-NMR spectroscopic analysis.[11] Coupling constants between the methine proton α to nitrogen and benzylic methylene protons are 0 and 9.9 Hz, indicating major product as **3**. On the other hand, those of minor product are 2.0 and 6.6 Hz, indicating minor product as **4**.

Heating of **3** at over the melting point established constant equilibrium to afford a mixture of **3** and **4** in a ratio of 40:1. In turn heating of **4** also provided a mixture in the same ratio. These thermodynamic behavior of the two isomers come from the isomerization of pivotal bond of biphenyl skeleton.[5] The preferred stability of **3** to **4** is attributed to the stability of the boat-chair conformation of dibenzocyclooctadiene of **3** rather than the boat-boat conformation of **4**.

Molecular mechanics calculations using Allinger's MM2 force field[12] indicate that **3** is stable more than **4** by 3 kcal/mol, consisting with the established equilibrium ratio. Dihedral angles of the calculated structure also support assignment of the structure on the basis of ^{1}H-NMR. Dihedral angles for the corresponding bond arrangement described above, -72 and +172 deg for **3** obtained by calculation and -90 and +24 deg for **4** are in good agreement with the coupling constants observed.

Preferred formation of **3** rather than **4** is attributed to stability difference between the two conformers of the intermediate of the reaction. The favorable intermediate **10**, in which seven membered ring maintains chair conformation, leads to **3** through spirodienone-phenol-type rearrangement. On the other hand, unfavorable intermediate **11**, in which seven membered ring maintains boat conformation, leads to **4**.

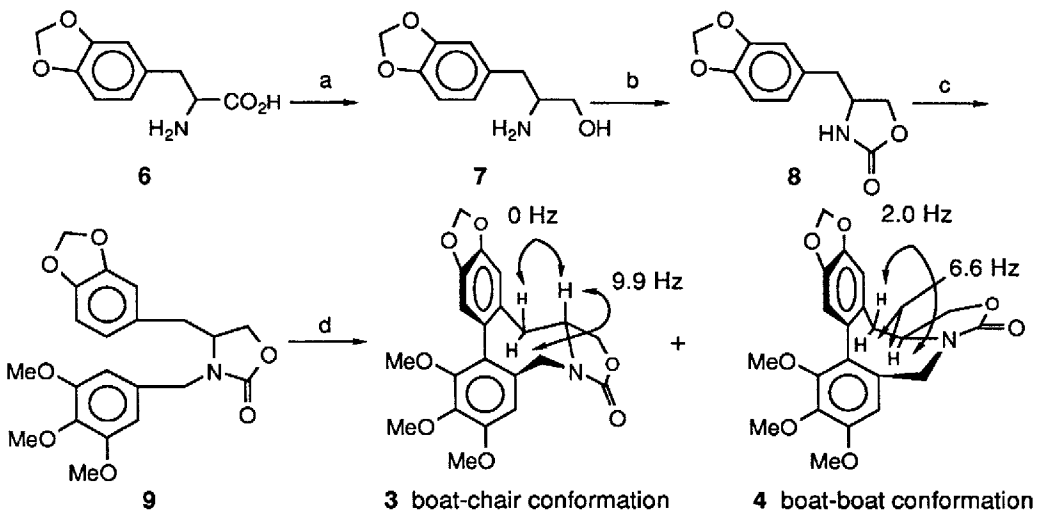
It is noteworthy that **3** was readily prepared in quantity from the known amino acid **6** in four steps in 57% overall yield.

Lithium aluminum hydride reduction of **3** and **4** provided the corresponding amino alcohols **12** (THF, reflux for 2 h, 82%, mp 88-90 °C) and **13** (THF, reflux 1.5 h, 23%), respectively. *N*-Formyl alcohol **15** was also obtained as a by-product by reduction of **4** (13%). The amino alcohol **12** was acetylated to **14** (Ac_2O/Py, rt for 2 h, 91%, mp 199-201 °C for hydrochloride).

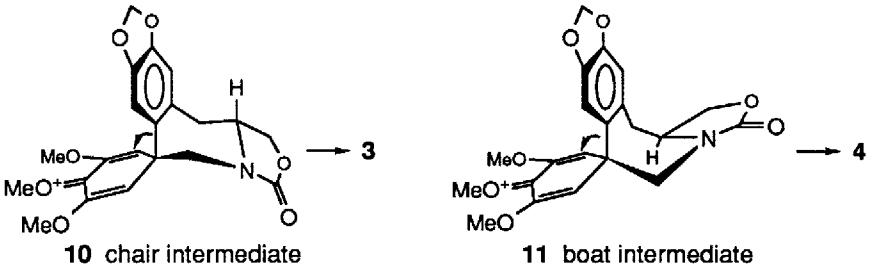
We are very pleased to find that **3** showed promising *in vitro* cytotoxic activity (ED_{50} <0.3 μg/ml against KB cell) as well as *in vivo* activity (T/C 137) against experimental tumor in P388 mouse. The activity is superior to those of natural lignan (-)-steganacin **1** as a leading compound.[6] On the other hand, marginal cytotoxicity was observed in the assay of **4** (47 μg/ml).

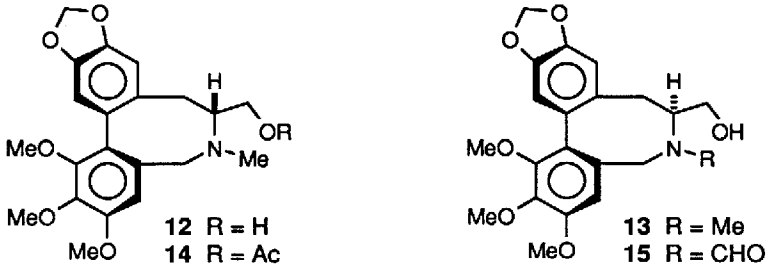
The amino alcohols **12**, **13**, and acetyl amine **14** showed the similar potency of activity (ED_{50} 1.1-1.65 μg/ml). It is quite interesting that *N*-formyl alcohol **15** showed an activity (ED_{50} <0.3 μg/ml) higher than the parent compound **4**.

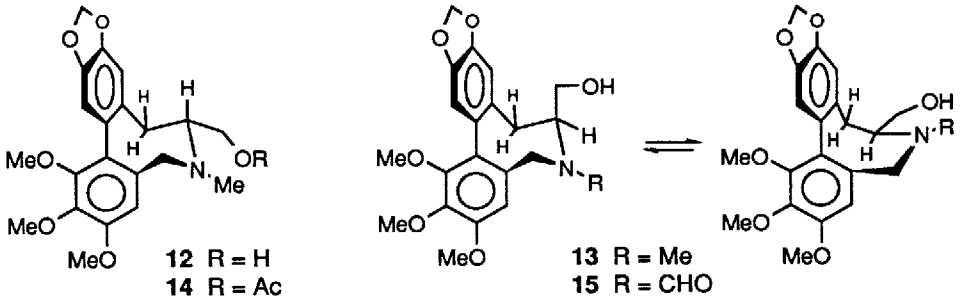
These variations in biological activity suggest that boat-chair conformation is an essential structure for biological activity. Boat-boat conformation of inactive **4** is converted to boat-chair



a) $LiAlH_4$/THF, reflux for 2 h, 70%; b) $OC(OEt)_2$-NaOEt/EtOH, reflux for 4 h, 89%; c) NaH-trimethoxybenzyl bromide/THF, reflux for 5 h, 96%; d) VOF_3/CH_2Cl_2-CF_3CO_2H, -42 °C for 3 h, 96% for **3**, 2% for **4**.







conformation by releasing rigidity through reductive opening of five membered ring, and then **13** and **15** showed activity. Furthermore, the presence of carbonyl group in a specific orientation is advantageous to show activity, and then **3** and **15** are of the most potent compounds.

We believe that conformation-activity relationships revealed by this work are useful in the development of the future anticancer drug.

Further study along this line including synthesis and evaluation of optically pure compounds is now in progress in our laboratories.[13]

Acknowledgement: The authors are grateful to Drs. Shigeru Tsukagoshi, Takashi Tsuruo, and Tazuko Tashiro, Cancer Institute, Japan for evaluation of antitumor activity.

References and Notes

1. "Anticancer Agents Based on Natural Product Models," ed. by J. M. Cassady and J. D. Douros, 1980, Academic Press.
2. Isolation. M. S. Kupchan, R. W. Britton, M. F. Ziegler, C. J. Gilmore, R. J. Restivo, and R. F. Bryan, *J. Am. Chem. Soc.*, **95**, 1335 (1973).
3. Synthesis and correction of absolute configuration of natural (-)-steganacin. K. Tomioka, T. Ishiguro, Y. Iitaka, and K. Koga, *Tetrahedron*, **40**, 1303 (1984).
   Recent synthesis. A. I. Meyers, J. R. Flisak, and R. A. Aitken, *J. Am. Chem. Soc.*, **109**, 5446 (1987), and references cited therein.
4. Biological activity. F. Zavala, D. Guenard, J.-P. Robin, and E. Brown, *J. Med. Chem.*, **23**, 546 (1980); P. B. Schiff, A. S. Kende, and S. B. Horwitz, *Biochem. Biophysi . Res. Commun.*, **85**, 737 (1978); R. W. J. Wang, L. I. Rebbun, and M. S. Kupchan, *Cancer Res.*, **37**, 3071 (1977).
5. K. Tomioka, H. Mizuguchi, T. Ishiguro, and K. Koga, *Chem. Pharm. Bull.*, **33**, 121 (1985).
6. ED_{50} (μg/ml) against KB cell. (-)-**1**: <0.3; (-)-**2**: <0.3; (-)-**5**: 8.8.
7. Unpublished result. For (-)-**1**, see reference 4.
8. S. Yamada, T. Fujii, and T. Shioiri, *Chem. Pharm. Bull.*, **10**, 680 (1962).
9. Satisfactory spectroscopic and analytical data were obtained for all new compounds described in this communication.
10. R. E. Damon, R. H. Schlessinger, and J. F. Blount, *J. Org. Chem.*, **41**, 3772 (1976).
11. 400 MHz ^{1}H-NMR ($CDCl_3$, TMS) δ: **3** : 2.48 (1H, dd, *J*=9.9, 13.9 Hz), 2.63 (1H, d, *J*=13.9 Hz), 3.63 (3H, s), 3.72-3.88 (2H, m), 3.90 and 3.91 (each 3H, s), 4.50-4.56 (1H, m), 4.64 (1H, d, *J*=14.3 Hz), 6.01 and 6.04 (each 1H, d, *J*=1.5 Hz), 6.72, 6.76, and 6.99 (each 1H, s); **4**: 2.43 (1H, dd, *J*=2.0, 14.3 Hz), 2.89 (1H, dd, *J*=6.6, 14.3 Hz), 3.54, 3.89, and 3.90 (each 3H, s), 4.02 (1H, m), 4.09 (1H, d, *J*=14.7 Hz), 4.09 (1H, dd, *J*=5.7, 8.9 Hz), 4.40 (1H, dd, *J*=8.6, 8.9 Hz), 4.78 (1H, d, *J*=14.7 Hz), 5.98 and 6.02 (each 1H, d, *J*=1.5 Hz), 6.59, 6.71, and 6.77 (each 1H, s).
12. Since biphenyl is not conjugated in the present system, usual parameters were used in the calculation.
13. The same strategy was applied to podophyllotoxin analogues. K. Tomioka, Y. Kubota, and K. Koga, accompanying paper.

(Received in Japan 15 March 1989)