Phytochemistry, Vol 27, No 11, pp 3663–3664, 1988
Printed in Great Britain

0031–9422/88 $3 00+0 00
Pergamon Press plc

A CAMPTOTHECIN DERIVATIVE FROM *NOTHAPODYTES FOETIDA*

Ritsuo Aiyama, Hisako Nagai, Kenichiro Nokata, Chigiru Shinohara and Seigo Sawada*
Yakult Central Institute for Microbiological Research, Yaho 1796, Kunitachi-shi, Tokyo 186, Japan

(*Received in revised form* 29 *April* 1988)

Key Word Index—*Nothapodytes foetida*; Icacinaceae, (20*S*)-camptothecin, (20*S*)-18,19-dehydrocamptothecin, 10-hydroxycamptothecin; 9-methoxycamptothecin

Abstract—A novel comptothecin derivative was isolated from the wood of *Nothapodytes foetida*. Its structure was elucidated by spectral data as (20*S*)-18,19-dehydrocamptothecin.

INTRODUCTION

(20*S*)-Camptothecin (**1**) having antitumour activity, was isolated first from the Chinese tree, *Camptotheca acuminata*. Decaisne (Nyssaceace) by Wall and co-workers in 1966 [for current review· 1]. In our studies of developing an antitumour drug, we previously reported the chemical modification of camptothecin [2–6] and their antitumour activity [7–9]. On searching for new synthetic sources, we isolated a new alkaloid, (20*S*)-18,19-dehydrocamptothecin from *Nothapodytes foetida* (Wight) Sleumer (formerly *Mapia foetida*) (Icacinaceae). We wish to describe the determination and characterization of this alkaloid

RESULTS AND DISCUSSION

The content of (20*S*)-camptothecin (**1**) in *N. foetida* is known to be larger (0.14–0.24% in dried plant) than that of *C acuminata* (*ca* 0.1% based on our measurement), and the former plant also contains 9-methoxycamptothecin (**4**) [11].

It is necessary to prepare a standard camptothecin sample for quantitative analysis However, the camptothecin purified by the usual procedure was only 96–98% pure On analysing HPLC, we succeeded in the base-line separation of camptothecin and the minor component on ODS-silica gel using eluent (a) 0.01 M aq. KH_2PO_4–MeCN–MeOH or eluent (b) aq. $HClO_4$–MeOH. They were inseparable by using other chromatographic conditions. Isolation of the component was carried out using HPLC 1 cm × 30 cm ODS column, eluent (a), repeatedly. From 1 kg of the dried plant material we obtained 1 9 mg of the component as pale yellow solid

This compound **2** showed a parent ion at *m/z* 346 in EIMS and HRMS at 346.0989 for $C_{20}H_{14}N_2O_4$, differing by two mass units from that of **1**. In its ^1H NMR spectrum, typical ABC spin coupling pattern attributed to a vinyl group appeared at δ 5.39 (1H, *d*, $J = 10.3$ Hz), δ 5.40 (1H, *d*, $J = 17.6$ Hz), and δ 5.89 (1H, *dd*, $J = 10.3$ Hz and 17.6Hz) instead of the ethyl group of **1**. Other ^1H NMR features of **2** were closly similar to those of **1**. Hydrogenation of **2** in the presence of palladium catalyst gave **1** quantitatively. In the CD spectrum, **2** showed a positive Cotton effect ($[\theta]_{241}$: +24400), and agreed with **1** (*S*-configuration :$[\theta]_{238}$: +59400). The CD spectrum of the hydrogenation product **1** coincided with that of naturally occurring **1**. Accordingly compound **2** was considered to be (20*S*)-18,19-dehydrocamptothecin [4(*S*)-4-hydroxy-4-vinyl-1H-pyrano [3′,4′,:6,7]-idolizino [1,2-*b*] quinolin-3,14-(4H,12H)-dione]

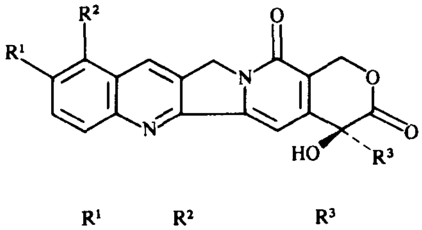
Compound **3** and **4** was also isolated from the same plant **3** was isolated first from *N foetida*. Camptothecin was said to be synthesized from tryptamine, derived from tryptophan, and iridoid secologanin in plants [11]. Compound **2** has a vinyl group, which seemed to originate from that of secologanin. The structural features of **2** gave an important clue to clarify the biosynthesis of camptothecin alkaloids via strictosamide [12]

EXPERIMENTAL

^1H NMR spectra were obtained using $CDCl_3$ at 400 MHz

Plant material *Nothapodytes foetida* (formerly *Mapia foetida*) was collected in the winter at Okinawa (Japan) by Dr T Shinzato (Department of Agriculture, Univ of Ryukyu), and identified by him The voucher specimen has been deposited in that Herbarium

Isolation of camptothecin derivatives Air-dried chipped stem wood (1 kg) of *N foetida* was extracted with MeOH (*ca* 20 l) This extract was condensed under red pres to *ca* 10 l The ppt (1 4 g) was then separated by filtration, and was washed with H_2O, MeOH, and EtOAc, successively It was passed through a silica gel column with $CHCl_3$–MeOH as eluent to yield compound **3** (25 mg) and **4** (270 mg) together with crude camptothecin fraction (*ca* 1 g) containing compound **2**. Crude camptothe-



	R¹	R²	R³
1	H	H	—CH_2Me
2	H	H	—$CH{=}CH_2$
3	—OH	H	—CH_2Me
4	H	—OMe	—CH_2Me

cin fraction (160 mg) was dissolved in DMSO (20 ml), and 200 × 100μ l portions were chromatographed repeatedly employing ODS column with the condition described below to afford a concentrated fraction of compound **2** Compound **2** was purified by rechromatography under same condition (1 9 mg)

LC conditions UV detector (wavelength 254 or 365 nm) Column·ODS (5 μm), 30 cm × 1 cm (YMC, Japan) or 40–45° with 0 01 M aq KH_2PO_4–MeCN–MeOH (3 1 1) or eluent The sample size was 100 μl (0 8 mg)/cycle

18,19-*Dehydrocamptothecin* **(2)** Pale yellow solid, MS (EI) *m/z* (rel int). 346 (51, M^+), 301 (100), 273 (57), 259 (11), 245 (14), 219 (18), 205 (11), HRMS *m/z* 346 0989 (for $C_{20}H_{14}N_2O_4$ = 346 0952) ^{1}H NMR ($CDCl_3$) δ 5 20 (*d*,1H,*J* = 16 1Hz, H-17), 5 33 (*br s*, 2H, H-5), 5 39 (*d*, 1H, *J* = 10 3 Hz, H-18), 5 40 (*d*, 1H, *J* = 17 6 Hz, H-18), 5 78 (*d*, 1H, *J* = 16 1 Hz, H-17), 5 89 (*dd*, 1H, *J* = 10 3 Hz, H-19), 7 69 (*dt*, 1H, *J* = 1 5 Hz and 8 1 Hz, H-10), 7 80 (*s*, 1H, H-14), 7 86 (*dt*, 1H, *J* = 1 5 Hz and 8 1 Hz, H-11), 7 96 (*d*, 1H, *J* = 8 1 Hz, H-9), 8 30(*d*, 1H, *J* = 8 1 Hz, H-12), 8 43 (*s*, 1H, H-7) CD $[\theta]_{241}$ +24400

Hydrogenation of compound **2** *to camptothecin* Compound **2** (1 85 mg) was dissolved in MeOH (50 ml) A part of the soln (10ml) was hydrogenated in presence of Pd-C as a catalyst under ambient H_2 with vigorous shaking to give camptothecin The reaction was completed within 20 min determined by HPLC CD spectrum of the filtrate of reaction solution was carried out directly

Acknowledgement—The authors are grateful to Dr T Shinzato (Department of Agriculture, University of Ryukyu) for the collection and identification of the plant, and thanks are also due to Mr T Makino for the skilful mass spectroscopy

REFERENCES

1 Cai, J -C and Hutchinson, C R (1983) *The Alkaloids* Vol 21, (Brossi, A., ed), p 101 Academic Press, New York
2 Miyasaka, T , Sawada, S and Nokata, K (1981) *Heterocycles* **16**, 1713
3 Miyasaka, T , Sawada, S and Nokata, K (1981) *Heterocycles* **16**, 1719
4 Yokokura, T., Miyasaka, T , Sawada, S , Nokata, K and Mutai, M (1981) *Proceedinq of the Japanse Cancer Association* The 40th Annual Meeting, October 1981, Sapporo
5 Sawada, S , Nokata, K , Miyasaka, T , Furuta, T , Yokokura, T and Mutai, M (1988) *Chem Pharm Bull* (in press)
6 Kunitomo, T , Nitta, K , Tanaka, T , Uehara, N , Baba, H , Takeuchi, M , Yokokura, T , Sawada, S , Miyasaka, T and Mutai, M (1987) *J Pharmacobio-Dyn* **10**, 481
7 Nitta, K , Yokokura, T , Sawada, S , Kunitomo, T , Tanaka, T , Uehara, N , Baba, H , Takeuchi, M , Miyasaka, T and Mutai, M (1985) *Recent Advances in Chemotherapy, Anticancer Section, 1 The Proceedings of the 14th international Congress of Chemother* Ishigami J , ed) p 29 Tokyo Univ Press
8 Nitta, K , Yokokura, T , Sawada, S , Kunitomo, T , Tanaka, T , Uehara, N , Baba, H , Takeuchi, M , Miyasaka, T and Mutai, M (1987) *Jpn J Cancer Chemother* **14** II, 850
9 Wang, Y , Chen, S -C and Ogawa, M , (1987) *Jpn J Cancer Chemother* **14** I, 1264
10 Govindachari, T R and Viswanathan, N (1972) *Indian J Chem* **10**, 453
11 Wani, M C and Wall, M E (1969) *J Org Chem* **34**, 1364
12 Hutchinson, C R , Heckendrof, A M , Daddona, P E , Hagaman, E and Wenkert, E (1974) *J Am Chem Soc* **101**, 3358

Phytochemistry, Vol 27, No 11, pp 3664 3667, 1988
Printed in Great Britain

0031 9422/88 $3 00 + 0 00

COMPONENTS FROM *SANTOLINA ROSMARINIFOLIA*, SUBSPECIES *ROSMARINIFOLIA* AND *CANESCENS*

M P Maqua, A C G Vines, E. Caballero, M. C Grande, M Medarde and I S Bellido*

Department of Organic Chemistry, Salamanca University, Salamanca, Spain

(*Received* 3 *February* 1988)

Key Word Index—*Santolina rosmarinifolia*, subspp *rosmarinifolia* and *canescens*, Compositae, terpenoids, sesquiterpenes, eudesmanes, coumarins, acetylenes

Abstract—Apart from other already known components, two-eudesmane-type alcohols, two coumarins and a new spiroketalenolether-type acetylene were isolated from *S rosmarinifolia*, subspp. *rosmarinifolia* and *canescens* The structures assigned were based on their spectral properties. The relative stereochemistries of the new acetylene at C-11 and that of the known spiranic acetylenes were assigned by NMR-NOE experiments

In previous work we studied the essential oils of *S rosmarinifolia*, L , subsp. *rosmarinifolia* [1] and *S rosmarinifolia*, subsp *canescens* (Lag.) Nyman [unpublished results] In the present paper we report the results of the study on components of the neutral non-volatile fraction of both,subspecies of *S rosmarinifolia*

From *S rosmarinifolia*, subsp *rosmarinifolia* we isolated 2-methyl-2,4-pentanediol (**1**), β-eudesmol (**2**), oplo-