ORGANIC LETTERS

2007
Vol. 9, No. 9
1813−1816

Cimicifugadine from *Cimicifuga foetida*, a New Class of Triterpene Alklaoids with Novel Reactivity

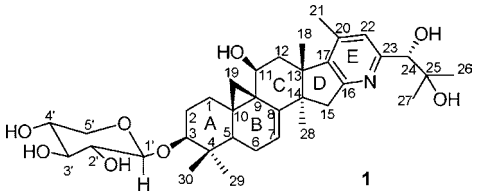
Chun Dan,[†,‡] Yan Zhou,[†] Deng Ye,[†] Shulin Peng,[†] Lisheng Ding,[*,†] Michael L. Gross,[§] and Samuel X. Qiu[*,§,||]

Chengdu Institute of Biology, Chinese Academy of Sciences, Chengdu 610041, People's Republic of China, Washington University, Campus Box 1134, One Brookings Drive, St. Louis, Missouri 63130, and Natural Products Drug Discovery, Herbstandard, Inc., 12305 New Avenue, Lemont, Illinois 60429

lsding@cib.ac.cn; samuelxqiu@gmail.com

Received March 3, 2007

ABSTRACT



1

An unprecedented triterpene alkaloid glycoside, designated cimicifugadine (1), with a pyridine ring incorporated to a cycloartane triterpenoid nucleus, was isolated from the roots of *Cimicifuga foetida*. Its structure was established on the basis of extensive spectroscopic measurements and chemical transformation with the absolute configuration at C-24 determined to be *S* by a modified Mosher method. It demonstrated a novel reactivity in mild acidic media whereby the cyclopropane ring is opened followed by the formation of two isomeric conjugated trienes.

The rhizome of several *Cimicifuga* (*Actaea*) species (family Ranunculaceae) is a source of a popular herbal medicine in China, Japan, and Korea.[1] Under the trivial name of "Shengma", it has been used as an antipyretic and analgesic agents since ancient times. In Europe and the United States, a dietary supplement made from the alcoholic extract of *Cimicifuga racemosa*, also called *Actaea racemosa* (black cohosh), has been reputed to reduce the frequency and intensity of hot flashes and other menopause symptoms.[1] Previous phytochemical studies have revealed that *Cimicifuga* species mainly contain several classes of constituents such as chromones, cinnamic acid derivatives, and 9,19-cyclolanostane triterpenoid glycosides, which are considered to be the bioactive components responsible for the biological activities of this herbal medicine.[2] Our extensive chemical investigations of *Cimicifuga foetida* (*Actaea cimicifuga*) led to the isolation of an unprecedented alkaloid glycoside, designated cimicifugadine (**1**), with a cycloartane triterpenoid skeleton possessing a pyridine ring.

The ethanol extract (1 kg) of the rhizomes of *C. foetida* was purchased from Jiahe Phytochem Co., Ltd. (Shanxi Province, China). It was partitioned with EtOAc and *n*-BuOH successively in a water bath (60 °C). The EtOAc extract (650 g) was subjected to repeated silica gel column chromatography and eluted with gradient solvent $CHCl_3$/MeOH (20:1−1:1) and finally a solvent mixture of petroleum ether and actone (2:1−1:1) to afford compound **1** (340 mg).

[†] Chengdu Institute of Biology.
[‡] Current address: College of Materials and Chemistry & Chemical Engineering, Chengdu University of Technology, Chengdu 610059, P. R. China.
[§] Washington University.
[||] Herbstandard, Inc.

(1) (a) *The Pharmacopoeia of Chinese People's Republic*; The Chemical Industry Publishing Hoouse: Beijing, China, 2005; p 50. (b) Nobuko, S.; Mutsuo, K.; Harukuni, T.; Teruo, M.; Fumio, E.; Hoyoku, N.; Masahiro, N.; Yohiro, S.; Kuo, H. L. *Bioorg. Med. Chem.* **2005**, *13*, 1403−1408. (c) Linda, S. E.; Masahito, S.; Xiao, D. H. *Breast Cancer Res. Treat.* **2004**, *83*, 221−231.

(2) (a) Nobuko, S.; Mutsuo, K.; Harukuni, T.; Yoshitaka, N.; Junko, T.; Hoyoku, N.; Akiko, K.; Genjiro, K.; Masahiro, N.; Yojiro, S.; Kuo, H. L. *Bioorg. Med. Chem.* **2003**, *11*, 1137−1140. (b) Masayuki, T.; Akiko, K.; Makio, S.; Genjiro, K.; Kenzo, K.; Ryuji, S.; Hye, S. K.; Yusuke, W. *Biol. Pharm. Bull.* **1998**, *21*, 823−828.

10.1021/ol070542m CCC: $37.00
Published on Web 03/29/2007

Table 1. ^{1}H and ^{13}C NMR Data of Cimicifugadine (**1**)[a]

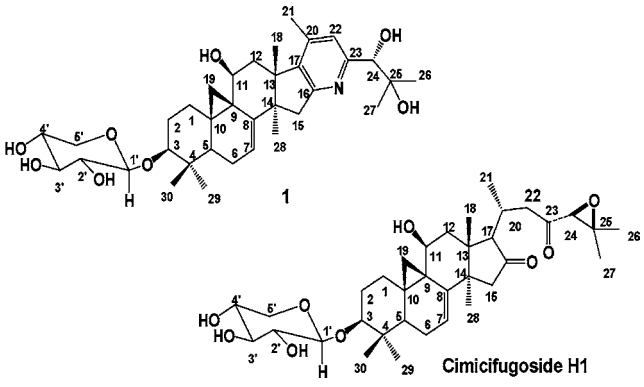
no.	δ_H*	δ_C*	δ_H	δ_C
1	2.76 (β) (d, 14.0), 1.75 (α)[b]	28.3 t	2.15 (β) (dd, 3.4, 13.5), 1.37(α)[a]	27.0 t
2	2.10 (α),[b] 2.43 (β)[b]	30.8 t	1.53 (α) (m), 1.80 (β) (m)	29.2 t
3	3.60 (α) (brd, 11.5)	89.3 d	3.14 (dd, 4.0, 11.5)	87.6 d
4		41.7 s		40.4 s
5	1.37 (α)[b]	44.9 d	1.12 (dd, 5.2, 12.2)	43.6 d
6	1.75 (β),[b] 1.96 (α)[b]	23.0 t	1.91 (α) (m), 1.67 (β) (t, 14.4)	21.7 t
7	5.32 (d, 7.7)	115.8 d	5.23 (dd, 6.4, 1.6)	114.4 d
8		147.3 s		146.3 s
9		28.7 s		26.9 s
10		30.4 s		28.9 s
11	4.60 (α) (d, 8.0)	64.0 d	4.11 (α) (m)	62.4 d
11-OH	nd		4.78 (d, 6.1)	
12	2.43 (β),[b] 3.06 (α) (t, 12.8)	45.4 t	2.80 (α) (dd, 9.5, 13.1) 2.01 (β) (dd, 2.7, 14.0)	43.5 t
13		49.6 s		48.2 s
14		52.3 s		50.9 s
15	2.83 (β) (d, 15.5), 3.23 (α) (d, 15.5)	45.0 t	2.95 (β),[b] 2.55 (α) (d, 15.3)	43.6 t
16		163.4 s		161.7 s
17		142.8 s		141.3 s
18	1.35 (s)	25.3 q	1.15 (s)	24.3 q
19	1.02 (d, 3.4), 2.01 (d, 3.4)	19.8 t	1.47 (d, 3.4) 0.75 (d, 3.4)	18.4 t
20		143.5 s		142.0 s
21	2.20 (s)	19.3 q	2.28 (s)	18.6 q
22	7.56[a]	123.7d	7.05 (s)	122.1 d
23		161.2 s		159.7s
24	5.05(β) (s)	80.5 d	4.26(β) (d, 5.4)	79.1 d
24-OH	nd		5.26 (d, 5.3)	
25		74.6 s		72.8 s
25-OH	nd		4.88 (s)	
26	1.51 (s)	26.9 q	0.93 (s)	25.9 q
27	1.64 (s)	28.6 q	1.04 (s)	27.2 q
28	1.06 (s)	29.6 q	0.85[b]	28.7 q
29	1.42 (s)	26.8 q	1.02 (s)	25.7 q
30	1.16 (s)	15.5 q	0.85[b]	14.4 q
1′	4.89 (d, 7.6)	108.4 d	4.16 (d, 7.6)	106.3 d
2′	4.04 (t, 8.2)	76.5 d	2.98[b]	74.3 d
3′	4.17 (t, 8.2)	79.5 d	3.08 (t, 8.5)	77.2 d
4′	4.22 (m)	72.1 d	3.27 (m)	70.1 d
5′	3.73 (t, 11.2) 4.32 (dd, 5.2, 11.2)	68.0 t	3.66 (dd, 5.2, 11.2) 3.02[b]	66.1 t

[a] NMR (600 MHz for ^{1}H and 150 MHz for ^{13}C) were recorded in either DMSO-d_6 or pyridine-d_5 (*). nd = peak not detected. [b] Overlapping multiplicity.

Compound **1**[3] afforded molecular ions at *m*/*z* 614 [M + H]$^+$ and 636 [M + Na]$^+$ in positive-ion ESI-MS. The odd molecular weight of 613 implies the existence of an odd number of nitrogen atom. Its akaloid nature is also evident from its positive response against Dragendorff's reagent. A molecular formula of $C_{35}H_{51}NO_8$ for **1** was deduced by HR-ESI-MS (found 614.3696 [M + H]$^+$, calcd 614.3687), corresponding to 11 double-bond equivalence (DBE). Its IR spectrum exhibited absorptions at 3405, 1631, and 1599 cm^{-1} owing to to hydroxyl groups and double bond stretches. The ^{1}H NMR spectrum (Table 1) showed the signals due to a typical cyclopropane methylene at δ 1.02 and 2.01 (each 1H, d, J = 3.4); seven *tert*-methyl groups at δ 1.06, 1.16, 1.35, 1.42, 1.51, 1.64 and 2.20 (each 3H, s); and an anomeric proton at δ 4.89 (1H, d, J = 7.6), in agreement with a structure of a cycloartane triterpene glycoside.

The DEPT spectrum exhibits a set of signals corresponding to a pentose moiety [δ_C 108.4 (d, C-1′), 79.5 (d, C-2′), 76.5 (d, C-3′), 72.1 (d, C-4′), and 68.0 (t,C-5′)], coincided with a spin-sysyem consisting of H1′−H2′−H3′−H4′−H$_2$5′, suggesting the presence of xylose or arabinose. The typical large coupling constants between H-1′ and H-2′ ($J_{H1'-H2'}$ = 7.6 Hz), H-2′ and H-3′ ($J_{H2'-H3'}$ = 8.2 Hz), and H-3′ and H-4′ ($J_{H3'-H4'}$ = 8.2 Hz) indicated that the protons at C-1′, C-2′, C-3′, and C-4′ have an axial, equatorial, axial, equatorial configuration, which means the hydroxyl groups at C-2′, C-3′, and C-4′ are in the α-, β-, and α-orientations, as found in β-D-xylopyranoside.[4] Thus, the sugar moiety must be either β-L-xylopyranosyl [^{1}C$_4$ chair conformation] or β-D-xylopyranosyl [^{4}C$_1$ chair conformation], with the latter being more favorable as it is a common component of the triterpene glycoside isolated from *Cimicifuga* plants, whereas the isolation of the former has not been reported. The glycon moiety of **1** was conclusively determined as β-D-xylopyranosyl by comparison of its ^{1}H and ^{13}C NMR data with those reported in the literatures which were found identical.[2] Further evidence for the absolute configuration of sugar was based on the optical rotation [α]$^{25}_D$ +90 (*c* 0.5, H$_2$O) determined for xylose obtained from acidic hydrolysis.

Other signals are for four oxygenated carbons resonating at δ 89.3 (d), 80.5 (d), 74.6 (s), and 64.0 (d) as well as seven olefinic carbons resonating at δ 163.4 (s), 161.2 (s), 147.3 (s), 143.5 (s), 142.8 (s), 123.7 (d), and 115.8 (d). All of the aforementioned evidence suggests that **1** is a 9,19-cycloartane triterpene xyloside based on the similarity of its NMR spectral profiles with those reported for other congeners.[4]



Comparing the ^{13}C NMR data with those of cimicifugoside H1[4] (Supporting Information), which was also isolated from the same source, we conclude that **1** possesses a similar structure to cimicifugoside H1 with respect to rings A, B, and C. The ^{1}H and ^{13}C signals arising from the second portion of the structure, however, differ profoundly from those of cimicifugoside H1 and do not match signals from any other structures reported thus far in the literature, suggesting a novel strcuture of compound **1**. Our efforts to make fine crystals from **1** failed which precluded the possibility to determine the structure directly by X-ray crystallography. Therefore, the structure determination of **1** will be achieved merely by interpretating of NMR spectroscopic data.

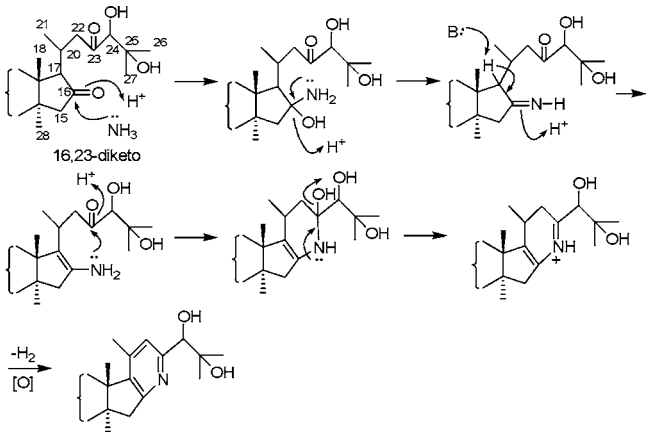
(3) Compound **1**: white powder; mp 246−248 °C; [α]$^{25}_D$ +13 (*c* 0.095, MeOH); IR (KBr) ν_{max} 3405, 2967, 1631, 1599, 1372, 1040, and 975 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; ESIMS *m*/*z* (rel int) 614 [M + H]$^+$ (100), 1249 [2M + Na]$^+$ (34), 636 [M + Na]$^+$ (16).

(4) (a) Mamoru, K.; Yoshinobu, A.; Nobuko, S.; Masahiro, N. *Chem. Pharm. Bull.* **1995**, *43*, 771−776. (b) Shigetoshi, Kadota, K.; Jian, X. L.; Ken, T.; Tsuneo, N. *Tetrahedron* **1995**, *51*, 1143−1166.

After deducting the contributions from the portion molecule consisting of rings A, B, and C, it is obvious that the second portion should possess two oxygenated carbons at δ 80.5 (d) and 74.6 (s) and four DBE to account for a total of 11. To accelerate the determination of substitution pattern of the second portion, the 1D and 2D NMR spectra were also recorded in DMSO-d_6 wherein the hydroxyl hydrogen atoms could be "frozen", therefore rendering them as "handles" for retrieving structure information particularly for quaternary carbons by interpretating the heteronuclear correlations from HMQC and HMBC. The signals due to hydroxyl groups at C-11, C-24, and C-25 positions are at δ 4.78 (11-OH), δ 5.26 (24-OH), and δ 4.88 (25-OH) based on the coupling with H-11 (δ 4.11) and H-24 (δ 4.26), respectively, in the COSY spectra. The vicinal relatonship of the latter two hydroxyl groups are evident from the HMBC correlations observed for H-24/***C***H_3-26, H-24/***C***H_3-27, and 24-OH/C-25; no noticeable correlations including 25-OH are detectable. Nevertheless, the presence of 25-OH was evident from the NOE correlation of 25-OH/CH_3-26 (27) and H24/CH_3-26 (27).

There are still one nitrogen atom and five olefinic carbons resonating at δ 163.4 (s), 161.2 (s), 143.5 (s), 142.8 (s), and 123.7 (d) to be assigned. The abnormally low field shift of the two olefinic carbons (δ 163.4 and 161.2) provides the evidence for the presence of a aromatic C=N functionality, which coincided with the UV absorptions at λ_{max} 221 (lg ϵ 4.85) and 271 nm (lg ϵ 4.69). Thus, to satisfy the unsaturation requirement and the aforementioned UV and NMR spectral intepretation, a pyridine ring including $C_{16}-C_{17}-C_{20}-C_{22}-C_{23}$ is proposed, portion connectivity of which was conclusively confirmed by the HMBC correlations as exemplified by H-15/C-16, H-15/C-17, and H-15/CH_3-28; CH_3-18/C-17, CH_3-21/C-17 and CH_3-21/C-22; H-22/C-17 and H-22/***C***H_3-21 (Scheme 1). The HMBC correlations were found to agree fully with the proposed structure. For example, the correlation between the signal at δ 4.89 (H-1′ of xylose) and δ 89.3 (C-3) establishes the linkage of the sugar to be at C-3. The position of the double bond (C_7-C_8) was assigned according to the HMBC correlations between δ 5.32 (H-7)/δ 52.3 (C-14), δ 1.96 (H-6a)/ δ 147.3 (C-8), 1.75 (H-6b)/C-8, and δ 1.06 (CH_3-28)/C-8.

Scheme 1. Proposed Biogenesis of Compound **1**



The relative stereochemistry of the aglycon of **1** was determined by interpreting NOESY data based on computer-generated lower energy conformation (Figure 1). The cor-

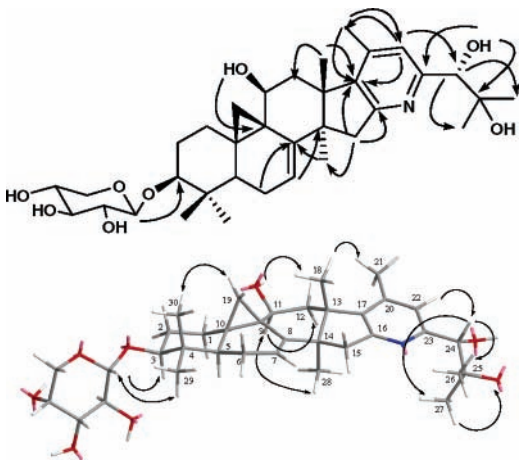


Figure 1. Key HMBC (→) and NOESY (↔) correlations of **1**.

relations of H-11 with CH_3-28 suggests that OH-11 is β-oriented. The NOE correlation pertinent to signals of protons and carbon from the portion of the molecule including carbons from rings A, B, C,and D, such as between H-1′/H-3, H-1′/CH_3-29; H_2-19/CH_3-18, and H_2-19/CH_3-30, indicate the configuration of rings A, B, C, and D are the same as for other congeners.[4] The absolute configuration of the stereogenic center C-24 is *S*, as determined by a modified Mosher method,[5] based on the analysis of the diagnostic $\Delta\delta$ values for like protons of (*S*)- and (R)-MTPA-**1** (Supporting Information). Thus, **1** was determined to possess the configuration of 3*S*,5*R*,9*R*,10*R*,11*S*,13*S*,14*R*,24*S*.

Compound **1** is an alkaloid glycoside containing a novel triterpene with an unprecedented architecture incorporating a pyridine ring in a cycloartane nucleus. To eliminate the possibility of compound **1** being an artifact formed during separation procedures or a contaminant, the freshly made EtOH extracts from several batches of plant materials *C. foetida* were analyzed by LC/MS, which confirmed the presence of **1** in all the tested samples, indicating that **1** is a naturally occurring product of *C. foetida*.

The biogenesis of **1** is of great interest given its unique structure. To account for the biogenetic origin of compound **1**, a plausible biosynthetic pathway was proposed as illustrated in Scheme 1, in which the pyridine ring originates from a coupling reaction between an amide group with the 16,23-diketo functionality, which is common in the cycloartane triterpenes from *Cimicifuga* species. Retention of the 24*S* stereochemistry of **1** further supports its close biogenetic relationship with other congeners as most of the cycloartane triterpenes isolated from *Cimicifuga* so far have the 24*S* configuration.

(5) Ohtani, I.; Kusumi, T.; Kashman, Y.; Kikisama, H. *J. Am. Chem. Soc.* **1991**, *113*, 4092−4096 and references cited therein.

During an attempt to obtain the aglycon by acidic hydrolysis, we discovered that **1** exhibits unique reactivity in mildly acidic media. With the exposure to 1 M HCl at 70 °C for 8 h, **1** becomes an inseparable, equimolar mixture (estimated on the basis of NMR spectrum) of two isomers (**1a**, **1b**)[6] with molecular formulas $C_{30}H_{41}NO_3$ (M_w = 463), as determined by HR-ESI-MS and the same DBE as **1**. Although the ^{13}C NMR spectrum of the two isomers are well-resolved, the significant overlap of ^1H NMR spectrum precluded the unambiguous assignment of the NMR signals. Nevertheless, several noticeable structural features can still be retrieved; for example, the NMR signals due to the cyclopropane methylene disappered, which indicates that the cyclopropane methylene is absent; the signals due to the xylose portion are also missing, suggesting removal of the sugar during the hydrolysis; the ^{13}C NMR data of **1a** + **1b** differ substantially from those of **1**, suggesting significant structure change must take place during the hydrolysis process. Acetylation of **1a** + **1b** affords an inseparable mixture of two isomers **1c** + **1d**,[7] with molecular formulas of $C_{34}H_{45}NO_5$ as determined by HR-ESI-MS. The two isomers originate from **1** by incorporating two acetyl groups. A closer comparison of the 1D and 2D NMR spectra of **1a** + **1b** with those of **1c** + **1d** reveals the compounds are structurally similar and that no other change other than acetylation of the two hydroxyl groups has occurred. Despite the complexity of the NMR spectra of **1c** + **1d**, its well-resolved feature allows unambiguous assignment of all the two sets of ^1H and ^{13}C NMR resonances by using a combination of COSY, HMQC, and HMBC experiments (Table 1S, Supporting Information). These isomeric compounds, **1c** + **1d**, exhibit several characteristic NMR spectral features, briefly, while the signals due to the pyridine ring remain unchanged, the protons assignable to the characteristic cyclopropane methylene (H_2-19) disappear and 10 signals of olefinic carbons become observable. The attachment of the two acetyl groups must be at C-3 and C-24 by the HMBC correlations observed between H-3/C=O, and H-24/C=O, which was further confirmed by the downfield shift of C-3 to δ 77.1 (**1c**) and δ 78.0 (**1d**), and C-24 to δ 81.5 (**1c** and **1d**) with respect to those of **1a** and **1b**. Obviously, the steric hindance between Me-26 and Me-27 prevents acetylation of 25-OH under the condition employed. As despicted in Figure 2, all of the correrations from COSY and HMBC are in agreement with the proposed strcuture.

As depicted in Scheme 2, we envision that **1** undergoes a structure conversion including a ring-B enlargement co-incident with the cleavage of the cyclopropane ring ($C_9/C_{10}/C_{19}$) and accompanied by the loss of H_2O involving 11-OH under mild acid media, followed by a double-bond rearrangement, to form two isomeric six-membered conjugated systems, **1a** and **1b**. Their abundance ratio is 1:1, as determined by LC/MS analysis. Although the cleavage of cyclopropane ring

(6) Compound **1a** and **1b**: yellow colloidal solid; ^1H and ^{13}C NMR data, see the Supporting materials; ESIMS *m/z* (rel int) 464 $[M + H]^+$ (100), 486 $[M + Na]^+$ (87), 949 $[2M + Na]^+$ (31).

(7) Compound **1c** and **1d**: brown colloidal solid; ^1H and ^{13}C NMR data, see Table 2; ESIMS *m/z* (rel int) 548 $[M + H]^+$ (100), 570 $[M + Na]^+$ (22).

(8) Narula, A. S.; Dev, S. *Tetrahedron* **1973**, *29*, 569−78.

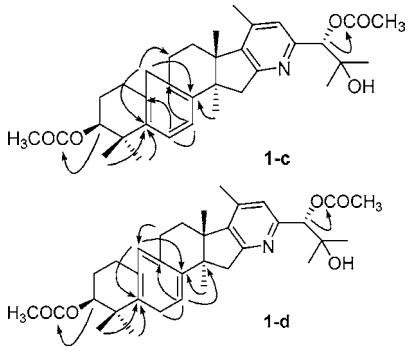
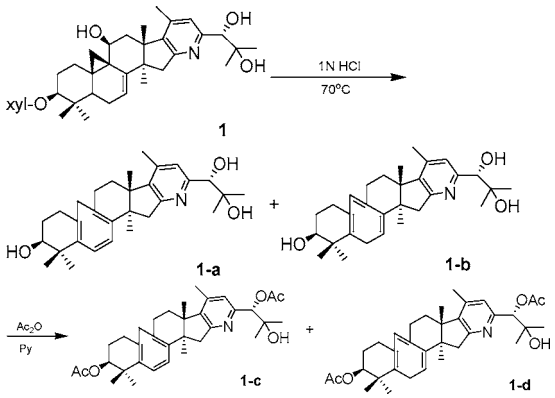


Figure 2. Key HMBC (→) and $^1H-^1H$ COSY (—) correlations of **1c** and **1d**.

can be induced by harsh treatment with CF_3CO_3H in CH_2Cl_2/K_2HPO_4,[8] we describe for the first time the formation of conjugated isomers involving the B ring under relatively mild acidic hydrolysis. This occurs presumably because there is a "neighboring group participation effect" of the proximate 11-OH group as shown in the lowest energy conformation, which faciliates formation of a carbocation and subsequent structure conversion cascade. This may be a new path to enhance chemical diversity of natural products and serve as a model for new synthesis strategies in organic chemistry.

Scheme 2. Acid Hydrolysis Followed by Acetylation of Compound **1**



Compound **1** exhibits moderate cytotoxicity against several human cancer cell lines at GI_{50} values of 12.5 μM (MCF-7, breast cancer), 9.0 mM (NCI-H460, non-small cell lung cancer), and 5.0 (K-562, leukemia) from a MTT assay (Supporting Information).

Supporting Information Available: 1D and 2D NMR spectral data of compounds **1**, **1a**, **1b** (with tentative assignment), **1c**, and **1d**; ^1H and ^{13}C NMR data of **1c** and **1d** in DMSO-d_6. 1D and 2D NMR spectral data of Mosher ester derivatives. Isolation of compound **1**. MTT cytotoxicity assay protocol. This material is available free of charge via the Internet at http://pubs.acs.org.

OL070542M