Wallifoliol, a Taxol Congener with a Novel Carbon Skeleton, from Himalayan Taxus wallichiana

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WALLIFOLIOL, A TAXOL CONGENER WITH A NOVEL CARBON SKELETON, FROM HIMALAYAN *TAXUS WALLICHIANA*[1]

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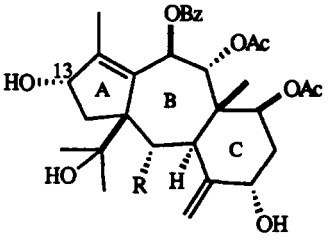
ABSTRACT.—A new taxoid, wallifoliol [**3**], has been isolated, along with five known taxoids (taxol, cephalomannine, 10-deacetylbaccatin III, brevifoliol, 2-acetoxy brevifoliol=taxchinin A) from extracts of the needles of Himalayan *Taxus wallichiana*. The structure of wallifoliol has been assigned primarily from nmr studies. Wallifoliol [**3**] is assigned a structure in which rings A and B of the taxane system have undergone putative rearrangements producing a novel skeleton. Wallifoliol is the first diterpene to be found in nature with this particular 5/6/6/6/4 ring system.

As part of a program searching for practical and biorenewable sources of taxol, 10-deacetylbaccatin III, and other potentially useful taxoid diterpenes (1), we isolated from less polar chromatography fractions brevifoliol [**1**], 2-acetoxy brevifoliol [**2**, subsequently shown by us to be identical to taxchinin A], and a modest amount of a new terpene (0.002%) which we have named wallifoliol and for which we propose a novel but biogenetically reasonable structure [**3**].

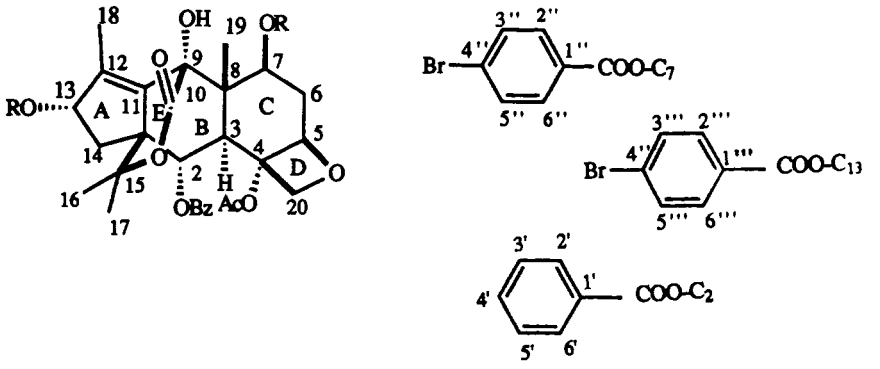
Wallifoliol [**3**], $C_{29}H_{34}O_{10}$, was isolated as an amorphous substance from a December 1990 collection of *T. wallichiana* Zucc. needles gathered in the vicinity of Darjeeling, India. Wallifoliol [**3**] migrated very close to taxchinin A (**2**, 2-acetoxy brevifoliol) and multiple chromatographies were required to separate them. The ir spectrum of wallifoliol [**3**] showed absorptions assignable to hydroxyl groups (3680, 3600, and 3440 cm^{-1}) and ester moieties (1720 cm^{-1}). The ^{1}H-nmr spectrum of **3** (Table 1) displayed signals comparable with those of brevifoliol-type compounds (2) with the important differences that ring-D oxetane signals [δ 4.20 (1H, d, J=8.5 Hz) and δ 4.66 (1H, d, J=8.5 Hz)] were seen, whereas ring-B signals attributable to H-9 and H-10 (normally found between δ 6.00–6.50 ppm) were missing. The ^{13}C-nmr spectrum (Table 1) showed signals for all twenty-nine carbons and their nature was deduced from the DEPT spectrum to originate from five methyls, three methylenes, five aliphatic methines, five aromatic methines, and eleven quaternary carbons, three of which were in carbonyl regions.



1 R=H
2 R=OAc

The HMBC spectrum (Table 1) of **3** revealed near-neighbor correlations between the signals at δ 164.7 and ortho aromatic protons (δ 7.96, 2H) (structural fragment **a**) and δ 170.2 with a methyl group (δ 1.72, s, 3H) (structural fragment **b**), respectively, indicating the presence of one benzoate and one acetate ester. Further, the ester carbonyl at δ 164.7 showed a long-range correlation with a proton signal at δ 5.78 (1H, d, H-2), which was further coupled to adjacent H-3 (δ 2.88, 1H, d) in its COSY spec-

[1]This manuscript is dedicated to Dr. Monroe Wall in recognition of his life-long contributions to natural products in general and the taxol field in particular.



3 R=H
4 R=*p*-BrC$_6$H$_4$CO-

trum (structural fragment **a**), thus requiring the benzoyl ester group to be at C-2, sharing this feature with many other known taxoids (3). There were no three-bond correlations to protons from the remaining carbonyl carbon signal (at δ 174.6) indicating that this group is neither an acetate nor a benzoate and is, indeed, attached to quaternary carbons at each end. Considering the molecular formula of wallifoliol [**3**] and subtracting the seven benzoate carbons and the two acetate carbons, twenty signals are left indicating that the remaining carbonyl is derived from the original taxoid skeleton carbons.

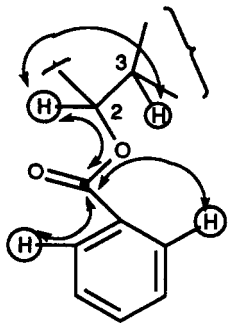
^{1}H-Nmr signals at δ 4.58, 2.18, 2.30, and 2.10 were assigned to H-13, H-14a, H-14b, and methyl-18, respectively, by their close correlation with the ring A signals of brevifoliol [**1**] (2,3) and the good agreement with these assignments in the HMBC nmr spectrum (Table 1) and ^{1}H-^{1}H COSY spectral correlations. Further analysis of the ^{1}H-COSY data revealed connectivities between δ 4.35 (H-7) and mutually coupled signals at δ 2.81 (H-6a) and 1.79 (H-6b), both with J=8 Hz (structural fragment **c**). The H-6a signal, but not that of H-6b, in turn showed coupling to H-5 (δ 4.85, J=8 Hz). These J values are quite different from those found in taxol (4) ($J_{5,6a}$=9.6 Hz; $J_{5,6b}$=2.3 Hz; $J_{6a,7}$=6.7 Hz; $J_{6b,7}$=10.9 Hz), indicating that the preferred conformation of the C ring is different from, and likely more distorted than, the flattened chair conformation of the C ring found in taxol and taxotere. These assignments also indicate that H-7 is not esterified and are further supported by long-range correlations between H-7 and methyl-19 hydrogens in the HMBC spectra.

Diesterification of **3** with *p*-bromobenzoic acid gave **4**. This experiment was performed partially in the hope that the resulting product would be crystalline and allow for X-ray structure determination, and also that newly introduced esters would be readily distinguishable from those already in place so facilitating deconvolution of the spectra. Unfortunately, this ester, though easily purified, was also amorphous. Its ^{1}H-nmr spectrum (Table 1) showed signals from two sets of additional (AA′BB′) aromatic protons at δ 7.63 (d), 7.94 (d) and 7.68 (d), 8.04 (d). The chemical shifts of H-7 and H-13 were observed at δ 5.60 and 5.80, having undergone downfield shifts of 1.25 and 1.22 ppm, respectively, as compared with δ 4.35 and 4.58 in wallifoliol, thus confirming the esterification and hydroxylation pattern.

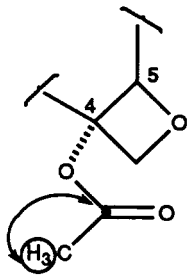
These considerations indicate that wallifoliol possesses most of the features of a taxoid of the brevifoliol subgroup except that signals attributable to H-9 and H-10 are missing and the corre-

TABLE 1. ^{1}H-, ^{13}C-, and HMBC Nmr Data of **3** and **4**.

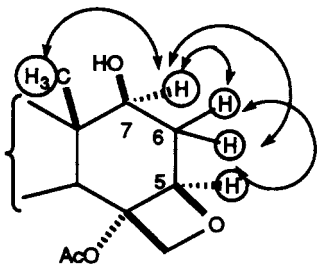
Position	3			4		
	δH	δC	HMBC	δH	δC	HMBC
1	—	59.9 s	H-2, H-3, H-14, H-16, H-17	—	60.7 s	H-2, H-3, H-14, H-16, H-17
2	5.78 (d, 12.0)	68.2 d	H-3, H-14b	5.84 (d, 11.9)	68.0 d	H-3, H-14b
3	2.88 (d, 11.9)	43.2 d	H-2, H-5, H-19, H-20	3.02 (d, 11.9)	42.6 d	H-2, H-5, H-19
4	—	80.5 s	H-3, H-5, H-6b, H-20	—	79.5 s	H-3, H-5, H-6b, H-20b
5	4.85 (d, 7.9)	84.2 d	H-6b, H-20a	4.88 (d, 6.5)	84.3 s	H-6b, H-20a
6	1.79 (dd, 7.9, 16.2)			1.86 (dd, 5.5, 16.7)		
	2.81 (m)	37.3 t	H-7	3.15 (m)	35.8 t	—
7	4.35 (t, 8.1)	71.0 d	H-3, H-5, H-6, H-19	5.60 (dd, 5.2, 9.6)	71.9 d	H-3, H-5, H-6b, H-19
8	—	48.3 s	H-2, H-3, H-6a, H-19	—	48.5 s	H-2, H-3, H-6a, H-7, H-19
9	—	84.9 s	H-3, H-7, H-19	—	82.9 s	OH-9, H-7, H-19
10	—	174.6 s	—	—	173.6 s	OH-9
11	—	131.0 s	H-13, H-14a, H-18	—	133.7 s	OH-9, H-13, H-14a, H-18
12	—	139.9 s	H-13, H-14a, H-18	—	136.7 s	H-13, H-14a, H-18
13	4.58 (t, 6.6)	79.6 d	H-14b, H-18	5.80 (t, 6.6)	81.9 d	H-14b, H-18
14	2.18 (m)			2.28 (dd, 5.5, 15.4)		
	2.30 (dd, 7.2)	37.1 t	H-2	2.49 (dd, 7.4, 15.4)	34.2 t	H-2
15	—	90.3 s	H-2, H-14, H-16, H-17	—	89.6 s	H-2, H-14, H-16, H-17
16	1.35 s	24.8 q	H-17	1.39 s	24.9 q	H-17
17	1.22 s	22.4 q	H-16	1.35 s	22.4 q	H-16
18	2.10 s	10.9 q	—	2.06 s	11.2 q	—
19	1.69 s	10.1 q	H-3, H-7	1.96 s	11.9 q	H-3, H-7
20	4.20 (d, 8.5)			4.22 (d, 8.4)		
	4.66 (d, 8.5)	74.2 t	H-3	4.66 (d, 8.4)	74.3 t	H-3, H-5
4-O-C=O Me	—	170.2 s	H of 4-O-C=O*Me*	—	169.0 s	—
	1.72 s	21.3 q	—	1.55 s	21.1 q	—
2-O-C=O Ph	—	164.7 s	H-2, ortho-H of Ph	—	164.9 s	H-2, ortho-H of Ph
7-O-C=O PhBr	—	—	—	—	164.1 s	H-7, ortho-H of PhBr
13-O-C=O PhBr	—	—	—	—	165.7 s	H-13, ortho-H of PhBr
aromatic						
2-OCO*Ph*						
1′	—	129.7 s	—	—	129.5 s	—
2′	7.96 (d, 7.1)	129.5 d	—	7.89 (d, 8.4)	129.4 d	—
3′	7.56 (m)	128.6 d	—	7.43 (m)	128.7 d	—
4′	7.60 (m)	133.6 d	—	7.58 (m)	133.5 d	—
5′	7.56 (m)	128.6 d	—	7.43 (m)	128.7 d	—
6′	7.96 (d, 7.1)	129.5 d	—	7.89 (d, 8.4)	129.4 d	—
7-OCO*PhBr*						
1″	—	—	—	—	128.3 s	—
2″	—	—	—	7.94 (d, 8.5)	131.1 d	—
3″	—	—	—	7.63 (d, 8.5)	132.0 d	—
4″	—	—	—	—	128.3 s	—
5″	—	—	—	7.63 (d, 8.5)	132.0 d	—
6″	—	—	—	7.94 (d, 8.5)	131.1 d	—
13-OCO*PhBr*						
1‴	—	—	—	—	128.3 s	—
2‴	—	—	—	8.04 (d, 8.6)	131.4 s	—
3‴	—	—	—	7.68 (d, 8.6)	132.0 s	—
4‴	—	—	—	—	128.3 s	—
5‴	—	—	—	7.68 (d, 8.6)	131.4 s	—
6‴	—	—	—	8.04 (d, 8.6)	131.4 s	—



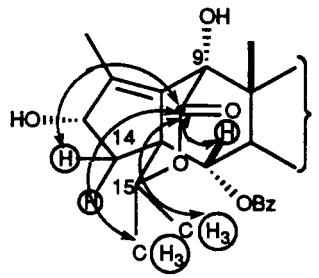
Fragment **a**



Fragment **b**



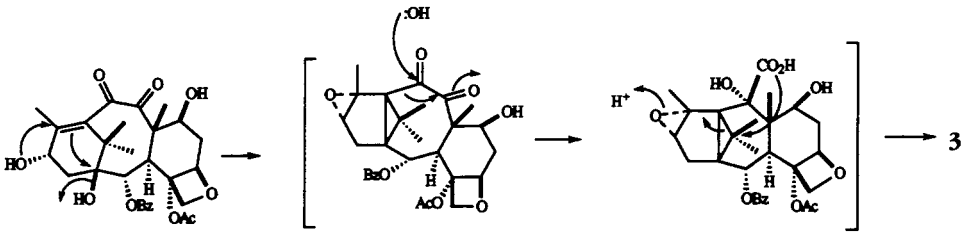
Fragment **c**



Fragment **d**

sponding carbons resonate at quite unusual positions. A δ-lactone assignment involving C-10 was suggested by the ir spectrum (1720 cm^{-1}) and, more importantly, by the chemical shift of C-15 which is found at δ 90.3! In brevifoliol, C-15 was found to resonate at δ 75.9 indicating attachment of an oxygen atom when compared with the peak at δ 44.8 seen with molecules possessing the baccatin III skeleton. The further downfield shift seen with wallifoliol would be consistent with its esterification but the only candidate available for such a carboxy-derived function would be C-9 or C-10. Extrusion of C-10 in the course of a benzilic acid rearrangement would not only allow for this but would easily accommodate the resonance of C-9 at δ 84.9, compared to δ 77.1 in brevifoliol which reflects its more deshielded environment. Identification of the signal at δ 90.3 as belonging to C-15 is strongly supported by near-neighbor four- and five-bond cross-peaks with methyl-16, methyl-17, H-14 and H-2 as well as by a smaller but definite upfield shift for C-1 (δ 59.9 from 62.4) (structural fragment **d**).

The only structure which readily accommodates all these features is **3**. One can readily imagine such a structure arising from a benzilic acid-type rearrangement of a taxoid precursor with carbonyl groups at both C-9 and C-10 [**5**] (Scheme 1). Such substances have recently been isolated from *Taxus* spp. (5–7). If this putative rearrangement product [**7**] were then to undergo the taxoid to brevifoliol rearrangement, but captured the new β-oriented carboxy group derived from C-10 instead of external H_2O, wallifoliol [**3**] would result. This possibility does not exist when C-10 and 11 have the normal taxane connectivities as in **6**. In this case, concerted epoxide opening accompanied by capture of H_2O, instead, is invoked and the brevifoliol series results. Now that the requisite precursor C-10,11 dicarbonyl analogs [**5**] have been found, it remains to see whether natural products such as **7**, which are as yet unknown, will turn up.



5 6 7

SCHEME 1

The pentacyclic 5/6/6/6/4 structure proposed for wallifoliol [**3**] is entirely consistent with its spectral and chemical properties as well as being biogenetically reasonable. The data, however, fall somewhat short of definitive proof because of the scarcity of material and the absence of closely precedent model substances. Subsequent collections and collections from other localities have yet to produce workable additional quantities. The search for this goes on.

Support for the idea that wallifoliol [**3**] is not an artifact generated by the extensive handling required for its isolation in pure form comes from its constant presence in tlc studies of early fractions and our failure to produce it by similar handling of other analogous extracts and fractions from other collections.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Nmr spectra were recorded with a Varian QE-300 or a Bruker 500 spectrometer in $CDCl_3$; δ are reported in ppm downfield to TMS as internal standard and *J* in Hz. Tlc was performed on Si gel (Merck, 0.25 mm), RP-18 plates and the solvent systems were hexane-Me_2CO (1.5:1), CH_2Cl_2-MeOH (19:1), C_6H_6-EtOAc (1:1), CH_2Cl_2-CH_3CN (2:1), and CH_3CN-H_2O-MeOH (1:0.8:0.4); detection: uv lamp and 10% H_2SO_4 reagent. Ir spectra were recorded with a Perkin-Elmer 1420. Uv spectra were recorded with a Hewlett-Packard 8450A.

PLANT MATERIAL.—*Taxus wallichiana* Zucc. (Taxaceae) was collected from Darjeeling, India in December 1990, by Dr. S.R. Vadapalli. A voucher specimen is on file at the Herbarium of Nagarjuna University, Nagarjunanagar, India.

EXTRACTION AND ISOLATION.—The air-dried powdered needles (1021 g) were extracted with hexane (room temperature, 24 h) followed by CH_2Cl_2-MeOH (1:1) (room temperature, 40 h). The hexane (31.1 g, 3%) and CH_2Cl_2-MeOH (131.6 g, 12.9%) extracts were concentrated *in vacuo*. The dark-green CH_2Cl_2-MeOH extract was partitioned between CH_2Cl_2 and H_2O. The organic layer was dried (anhydrous Na_2SO_4), filtered, and evaporated to dryness (53.1 g, 5.2%). The extract was subjected to cc on Si gel (23 g of Si gel/1 g of extract) and 387 fractions of 20 ml were eluted with a polarity gradient solvent system starting with hexane-Me_2CO (4:1). Fractions 15–35 showed similar tlc patterns and were combined. These, on concentration, gave 2.3 g (0.22%) of mixture. This on rechromatographing (Si gel) using C_6H_6-EtOAc (1:3) yielded 1.0 g (0.098%) of a mixture. Further chromatographic separation using CH_2Cl_2-MeOH (15:1) yielded 0.6 g (0.058%) of purified material. Brevifoliol [**1**] and a mixture of **2** and **3** were obtained after rechromatography (Si gel) using CH_2Cl_2-CH_3CN (2:1). Final purification by prep. tlc on RP-18 plates with CH_3CN-H_2O-MeOH (1:0.8:0.4) afforded **3** (19.5 mg 0.002%) and **2** (34.6 mg, 0.0034%) in pure form.

Wallifoliol [**3**].—Amorphous; $[\alpha]^{25}D$ −10.8° (*c*=0.65, MeOH); uv λ max (MeOH) nm (log ε) 229 (4.26); ir ν max (KBr) 3680, 3600, 3440, 3040, 1720, 1600, 1370, 1240, 1170, 1100, 1050, 1020, 970, 890 cm^{-1}; fabms *m/z* $[M+Na]^+$ 565 (49), $[M+1]^+$ 543 (100), 525 (22), 465 (15), 343 (14), 325 (14); for ^1H-, ^{13}C-, and HMBC nmr data, see Table 1.

Esterification of **3**.—Wallifoliol **3** (9.4 mg) in DMAP (12.3 mg) was added to the CH_2Cl_2 solution of *p*-bromobenzoic acid (19.4 mg) and DCCI (25.2 mg). The reaction mixture was stirred for 20 h at room temperature and the precipitated dicyclohexyl urea was filtered and the filtrate was concentrated. The residue was dissolved in CH_2Cl_2, washed with 1N HCl, saturated $NaHCO_3$, and H_2O. The CH_2Cl_2 solution was evaporated to dryness and compound **4** (7.1 mg, 76%) was purified on RP-18 prep. tlc using CH_3CN-H_2O-MeOH (1:0.2:0.8). For ^1H-, ^{13}C-, and HMBC nmr data, see Table 1.

ACKNOWLEDGMENTS

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