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TAXANES FROM THE BARK OF *TAXUS BREVIFOLIA**

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Key Word Index—*Taxus brevifolia*; Taxanes; Taxaceae.

Abstract—Large-scale processing of the chloroform extract of the bark of *Taxus brevifolia* by reversed phase column chromatography on C-18 bonded silica using acetonitrile/water mixtures, gave a taxane-rich fraction which emerged just after the elution of paclitaxel. Re-chromatography of this fraction yielded 15 taxane constituents, many of which were obtained in crystalline from, and variously belonging to the 11(15 → 1)-abeotaxane group, taxinine J group, brevifoliol group and those with an oxygenation pattern at the 14 position. Five of these were new and several others were isolated for the first time from this source. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

In view of the demonstrated clinical effectiveness of paclitaxel **1** [1] in ovarian, breast and other carcinomas [2, 3], there has been an intensive effort to search for other members of the taxane group, which may either be directly active, or serve as precursors for the semi-synthesis of other active analogues. An example of such an effort was the discovery of the 13-acetyl-9-dihydrobaccatin III [4] which led to the semi-synthesis of the active analogue: 9-dihydropaclitaxel [5]. In our continuing studies on the use of reversed phase column techniques on the extracts of *Taxus brevifolia* for the purpose of isolation of paclitaxel and several of its analogues [6, 7], we had a chance to examine some of the fractions for additional taxane constituents. A relatively low polarity taxane-rich fraction which emerged from this column immediately following paclitaxel was re-chromatographed, and a number of crystalline taxane constituents were isolated from it and characterized. Of the 15 compounds so separated, seven belonged to the 11(15 → 1)-abeobaccatin VI group, with four being new members. Four others belonged to the 11,4(20)-taxadiene group represented by taxinine J; two with the oxygen substitution at C-14, as seen in taiwanxan; one, a new member of the brevifoliol group, and one, identified as baccatin VI. A discussion of the isolation and structural characterization of these components is presented in this communication.

The initial recognition of these taxanes was through the use of TLC, with visualization by UV as well as by charring with dilute sulfuric acid spray, whereby they produced characteristic dark greenish blue, or gray-brown spots. Separation and purification involved, for the most part, the usual two column sequence using normal phase silica: the first for the gross separation and the second for fine separation. Solvent mixtures consisting of acetone (or, alternatively, dichloromethane, ethyl acetate or benzene) and ligroin were used, and the components crystallized where possible.

The initial silica column gave the four major components **2**, **3**, **4** and **5**, which were readily obtained as crystalline solids, with **3** being present in the highest yield. The mother liquors from **2**, **3** and **4** were combined with the even faster moving components which came from the same column, and re-chromatographed using one or the other of the combination of solvents listed above. It was also observed that the R_f sequence (order on the TLC) was different depending on the solvent system used, and this behavior was helpful in their subsequent column separations. Thus, compounds which co-eluted in one solvent mixture separated when one of the other solvent mixtures was used. It was thus possible to isolate **10**, **12**, **13**, **14** and **16**, with all except **10** being obtained as crystalline solids. Another fraction from this column, which also appeared as a single entity on TLC, but was found to be a mixture by its NMR spectrum, was difficult to resolve. However, one of the components of this mixture crystallized readily to give **11**, which thus could be separated and obtained pure. The NMR spectrum of the remaining mixture suggested the presence of

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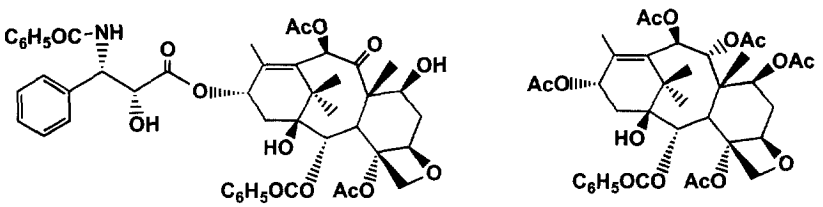


1 16

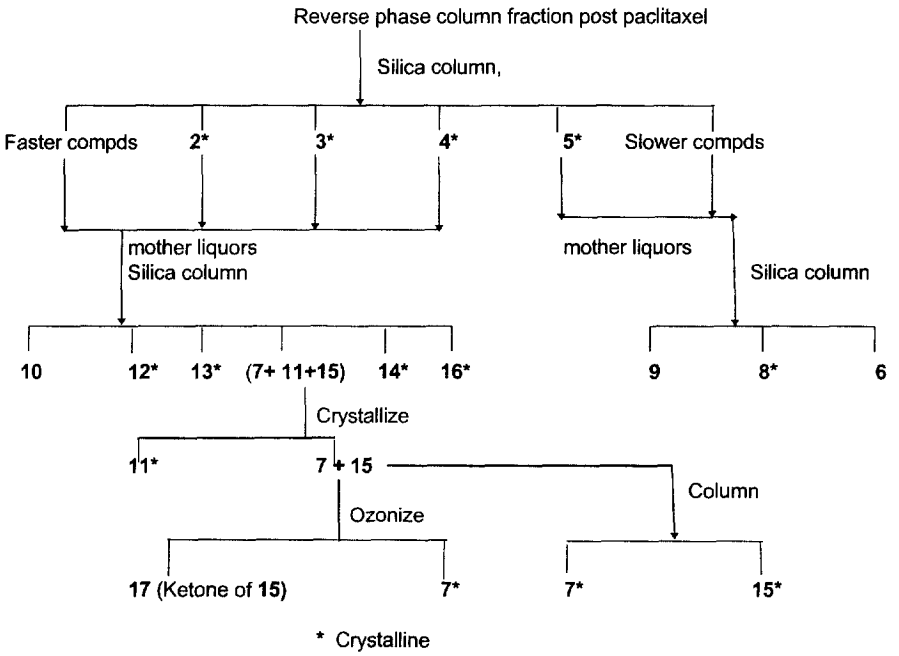
Fig. 1. Structures **1** and **16**.

possibly two taxane components, occurring in a roughly 1:1 ratio, with one containing a 4/20 double bond and the other an oxetane ring at this position. This mixture was subjected to ozonization which made possible the isolation of **7**, while **15** underwent conversion to the corresponding ketone **17**. Subsequently, by using chromatography with benzene/ligroin mixtures, **7** and **15** could be separated directly also.

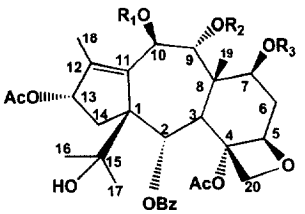
Analogously, from the original silica column, after the bulk of the slower moving compound **5** was removed by crystallization, the mother liquors, combined with the still slower moving fractions from the same column were re-chromatographed, and compounds **6**, **8** and **9** isolated in pure form. Scheme 1 shows the steps described above and the location of the fifteen components.

The ^{1}H and ^{13}C NMR spectra of compounds **2**–**8**, showed that they possess the 11(15 → 1)-abeotaxane structure, by having an oxetane ring (pair of doublets at δ 4.20 and 4.50), and a 5-membered ring A (13-H on a carbon containing an ester function, showing a signal at δ 5.70, and a C-15 signal appearing at δ 74.3–75.1 ppm). Of these, **2**, **3** and **4** were found to be already known, being isolated earlier by Chu *et al.* [8] as amorphous solids, and were given structures based on a 6-membered A-ring. Fuji *et al.* [9] reported the isolation of **2**, which they named taxchinin C, as a crystalline solid and assigned its structure as **2**, in which the A-ring was 5-membered. Based on X-ray crystallographic studies, Chu *et al.* [10] reassigned structures for **2**, **3** and **4**, based on a 5-membered A-ring, i.e. with the 11(15 → 1)-abeotaxane skeleton. Compounds **5**–**8** are presently shown to be four new members of this unusual subgroup of abeobaccatins VI, of which at least 21 members are known, including these four (11, 12).

The structural assignments for compounds **5** and **8** were made both by spectral means (^{1}H, ^{13}C, COSY, HETCOR and HMBC), and chemical reactions. The ^{1}[H] and ^{13}[C] spectral assignments of **6**–**8** are given in Table 1. For example, compound **6** on acetylation gave **3**, and on benzoylation, gave **7**. Mild basic hydrolysis of **3** produced **6**. Likewise, acetylation of **8**

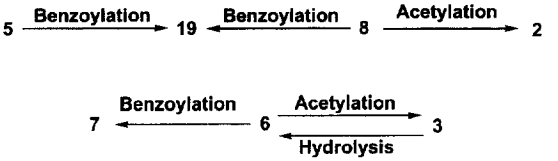


Scheme 1.



	R_2	R_3
2:	Bz	Ac
3:	Ac	Bz
4:	Ac	Ac
5:	Bz	Bz
6:	Ac	Bz
7:	Ac	Bz
8:	Bz	H
18:	Bz	Bz
19	Bz	Bz

Fig. 2. Structures **2**–**8**, **18** and **19**.



Scheme 2.

gave **2**, and benzoylation yielded a benzoate **19**, which was also produced by the benzoylation of **5**. Thus these conversions which are shown in Scheme 2, indicate not only the inter-relationships between **5**–**8**, but also their relationship to compounds **2** and **3**, of established structures, and leading to the conclusion that all of these compounds contain a benzoate at C-2 and an acetate at C-13. Thus, the final structural elucidation of **5**–**8** will require the correct placement of the acetate, benzoate and hydroxyl functions at 7, 9, and 10 positions. Since compounds **2**, **3** and **4**, [10] as well as **21** and **24** ([16], see further) are all of known structures, one can start with these in deciding on the specific assignments.

The ^{1}H NMR spectrum of **5** gave rounded peaks in $CDCl_3$ at room temperature, thus indicating the presence of a rotameric equilibrium, although one could infer the presence of one hydroxyl, three benzoyl, and two acetyl groups. The spectrum taken

Table 1. ^{1}[H] and ^{13}[C] NMR spectra of compounds **6**, **7** and **8**

H#	Compd. 6	Compd. 7	Compd. 8	C#	Compd. 6	Compd. 7	Compd. 8
2	6.26 (*br d*, 7.5 Hz)	6.49 (*d*, 8.0 Hz)	6.51 (*d*, 7.8 Hz)	1	67.5	75.6	68.1
3	3.12 (*d*, 7.5 Hz)	3.21 (*d*, 8.0 Hz)	3.08 (*d*, 7.8 Hz)	2	66.2	68.3	68.4
5	5.00 (*d*, 7.8 Hz)	5.02 (*d*, 7.2 Hz)	4.99 (*d*, 8.4 Hz)	3	44.7	44.6	44.4
6α	2.64 (*m*)	2.69 (*m*)	2.70 (*m*)	4	79.2	79.2	79.5
6β	2.05 (*m*)	2.08 (*m*)	1.86 (*m*)	5	84.6	84.6	84.7
7	5.72 (*t*, 7.2 Hz)	5.90 (*t*, 8.4 Hz)	4.60 (*t*, 8.1 Hz)	6	34.8	34.9	36.6
9	5.84 (*br d*, 9.8 Hz)	6.27 (*d*, 10.5 Hz)	6.68 (*d*, 11.1 Hz)	7	70.7	70.4	71.5
10	4.76 (*br d*, 9.8 Hz)	6.73 (*d*, 10.5 Hz)	6.74 (*d*, 11.1 Hz)	8	43.5	44.0	44.3
13	5.72 (*br t*, 7.2 Hz)	5.71 (*t*, 7.2 Hz)	5.74 (*t*, 7.8 Hz)	9	79.4	76.5	79.1
14α	2.30 (*m*)	2.44 (*dd*, 14, 7.2 Hz)	2.46 (*dd*, 14, 6.9 Hz)	10	68.6	68.9	67.8
14β	1.90 (*m*)	1.95 (*m*)	2.02 (*m*)	11	140.0	135.9	136.5
16	1.11 (*s*	1.16 (*s*	1.26 (*s*)	12	142.8	148	147.2
17	1.18 (*s*	1.18 (*s*)	1.26 (*s*)	13	79.4	78.7	78.6
18	1.92 (*s*)	2.04 (*s*)	1.88 (*s*)	14	36.4	36.7	36.9
19	1.83 (*s*)	1.89 (*s*)	1.76 (*s*)	15	74.4	75.1	74.3
20α	4.49 (*d*, 7.8 Hz)	4.54 (*d*, 7.8 Hz)	4.47 (*d*, 7.8 Hz)	16	27.8	27.8	27.9
20β	4.17 (*d*, 7.8 Hz)	4.17 (*d*, 7.8 Hz)	4.17 (*d*, 7.8 Hz)	17	25.5	25.5	25.6
(Ac)	1.40, 2.18, 2.19	1.03, 2.18, 2.22	2.17, 2.22	18	11.3	12.1	11.6
				19	12.8	12.8	11.8
				20	76.3	74.6	75.9
				Ac/Me	22.0, 21.2, 20.4	22.0, 21.2, 19.8	21.0, 22.0
				Ac/CO	171.7, 170.9, 169.0	170.6, 170.1, 169.0	169.2, 170.6
				Ph/CO	166.0, 165.3	165.9, 165.1, 163.8	165.8, 165.1, 164.9

Table 2. ^{1}H NMR comparison of 5(18 and 19), 6,7 and 8 with the known members

pd#	H-2	H-7	H-9	H-10	H-13
2	$6.57_{(Bz)}$	$5.70_{(Ac)}$ (0.17)	$6.58_{(Bz)}$ (0.45)	$6.78_{(Bz)}$ (0.47)	$5.74_{(Ac)}$
3	$6.40_{(Bz)}$	$5.82_{(Bz)}$ (0.29)	$6.09_{(Ac)}$ (−0.04)	$6.47_{(Ac)}$ (0.16)	$5.72_{(Ac)}$
4	$6.46_{(Bz)}$	$5.63_{(Ac)}$ (0.10)	$6.30_{(Ac)}$ (0.17)	$6.62_{(Bz)}$ (0.31)	$5.70_{(Ac)}$
18	$6.53_{(Bz)}$	$5.89_{(Bz)}$ (0.36)	$6.49_{(Bz)}$ (0.36)	$6.61_{(Ac)}$ (0.30)	$5.74_{(Ac)}$
19	$6.61_{(Bz)}$	$5.97_{(Bz)}$ (0.44)	$6.64_{(Bz)}$ (0.31)	$6.87_{(Bz)}$ (0.56)	$5.74_{(Ac)}$
6	$6.26_{(Bz)}$	$5.72_{(Bz)}$ (0.19)	$5.84_{(Ac)}$ (−0.29)	$4.76_{(H)}$	$5.71_{(Ac)}$
7	$6.49_{(Bz)}$	$5.90_{(Bz)}$ (0.37)	$6.27_{(Ac)}$ (0.14)	$6.73_{(Bz)}$ (0.42)	$5.71_{(Ac)}$
8	$6.51_{(Bz)}$	$4.60_{(H)}$	$6.68_{(Bz)}$ (0.55)	$6.74_{(Bz)}$ (0.36)	$5.74_{(Ac)}$
21	$6.09_{(Bz)}$	$4.23_{(H)}$	$4.35_{(H)}$	$4.56_{(H)}$	$5.72_{(Ac)}$
24	$6.36_{(Bz)}$	$5.53_{(Ac)}$	$6.13_{(Ac)}$	$6.31_{(Ac)}$	$5.67_{(Ac)}$

at −20 C, confirmed the presence of two conformers in a 2:3 ratio. Acetylation of **5** gave one monoacetate **18**, and benzoylation, the monobenzoate **19**, both of which gave normal ^{1}H NMR spectra, which were used in place of that of **5**.

A comparison of the ^{1}H NMR spectra of the known compounds listed above (see Table 2) clearly indicated that the position of the H-13, with a CH–OAc remained virtually constant, (δ 5.7±0.03), while that for the H-2, with a CH–OBz was within a relatively narrow range, (δ 6.25±0.3). Depending on the presence of any additional benzoate groups, the signals for H7, H-9 and H-10 varied over a wider but predictable range. Thus, starting with **24** which has acetates at each of these positions as a reference, the presence of a CH–OBz is found to produce a further down-field shift of 3.3–0.4 ppm, as well as causing an additional down-field shift of 0.1–0.15 ppm for the CH–OAc. The well-known fact that H-10, being allylic is the most down-field of these signals is also to be taken into account, as well as the effect of any hydroxyls in the region. Thus, based on the spectra of **18** and **19** (acetate and benzoate respectively of **5**), one can arrive at a substitution pattern for **5** of 7-CH–OBz, 9-CH–OBz and 10-CH–OH.

Compound **6** has one hydroxyl, two benzoate and three acetyl groups. From its relationship to **7** and **3** one can infer the substitution pattern of 7-CH–OBz, 9-CH–OAc, 10-CH–OH.

Compound **7** has three acetate and three benzoate groups, and based on the above discussion, a substitution pattern of 10-CH–OBz, 9-CH–OAc, 7-CH–OBz can be deduced. Compound **8** with its one hydroxyl, three benzoate and two acetate groups is isomeric with **5**. The spectral comparison permits an assignment of 10-CH–OBz, 9-CH–OBz, 7-CH–OH for **8**.

The above assignments were confirmed by a long range HETCOR (HMBC) spectra taken on **18**, **7** and **8**.

In compound **18**, one of the signals of Ph–CO (δ 165.2) interacted with that of H-7. The second signal of Ph–CO (δ 165.7) showed an interaction with that at δ 6.5, where the chemical shifts of H-2 and H-9 also overlap. The third Ph–CO did not show interaction. Since C-2 carries the benzoate as determined from the inter-conversions, the benzoate (δ 165.7) can be assigned to C-2. The choice between the C-9 and C-10 for the third benzoate was made from the oxidation reactions (see below). Interaction was also seen for one of the acetyl carbonyls (δ 167.8) with the CO–CH_3 signal at δ 1.56.

In **6**, the assignment made earlier for the C-16, 17, 18 and 19 and the three CO–CH_3 were based on the ^{13}C NMR spectroscopy analogy with reported compounds of the same class. The currently reported assignments are based on the HETCOR spectra.

The HMBC spectrum of **7** showed a correlation between the Ph–CO (δ 165.9) and the *o*-protons of the Ph–CO (δ 8.04), the H-2 signal (δ 6.50), the *o*-Ph–C (δ 129.7) and the *p*-Ph–C (δ 133.5). The Ph–CO signal at δ 165.1 correlated with the H-7 (δ 5.90) and with *o*-Ph–H (δ 8.12), which in turn, coupled with *o*-Ph-C (δ 129.7) and *p*-Ph-C (δ 132.8). In addition, correlation was seen between H-18 (δ 2.04) and the C-11 (δ 135.9), C-12 (δ 147.9) and C-13 (δ 78.7). Similarly, H-19 showed long range coupling to the C-3 (δ 44.6), C-7 (δ 70.4) and C-9 (δ 76.5). Lastly, interaction of the Ac–CO was seen with a Me Signal at δ 1.03, which was assignable to a CO–Me (δ 19.8) by the HETCOR spectrum.

The HMBC spectrum of **8** showed correlations of all the three Ph–CO signals with their corresponding proton signals on the taxane skeleton: δ 165.8 vs H-2, δ 164.9 vs H-9 and δ 165.1 vs H-10, thereby locating the benzoate groups at these sites. The spectrum also showed a correlation between the H-13 signal with one of the two Me–CO signals resonating at δ 21.0. Interactions were also seen between H-18 and C-11, C-12 and C-13, as well as that between H-19 and C-3, C-7 and C-9.

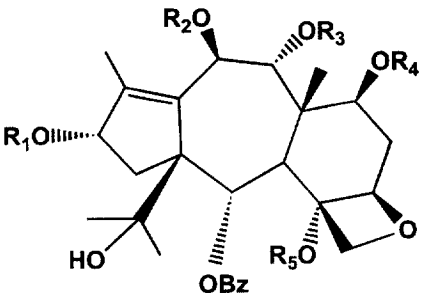
From the preceding HMBC spectral data, it was possible to correct the earlier assignments for the acetyl groups and the methyl groups (C-18 and 19). With regard to the acetyl signals, considerable variation was observed, which could be attributed to the benzoate esters, their number and location. For example, in compounds **2** (7-Ac, 9,10-Bz), **3** (7-Bz, 9,10-Ac) and **4**

(7,9-Ac, 10-Bz), one of the acetyl signals appears at δ 1.84, 1.86 and 1.77, respectively. Even more unusually, one of the acetate signals of compounds **18** (7,9-Bz, 10-Ac), **6** (7-Bz, 9-Ac, 10-OH) and **7** (7,10-Bz, 9-Ac) appears at 1.56, 1.40 and 1.03 ppm, respectively. In the case of the last compound, one might speculate the methyl group of the 9-Ac may be within the ring current of the two neighboring benzoates at C-7 and C-10.

Since the spectrum of **5** could only be studied in the form of its acetate **18**, it is necessary to locate the hydroxyl in **5**. Oxidation of **5** was carried out with Jones reagent to the ketone **20**. The ^{13}C NMR spectrum showed the signal for the 10-keto function at 191.8 ppm, in line with its expected position for an $\alpha\beta$-unsaturated ketone, while the ketone at C-9 is generally seen at 207–209 ppm. The signal for C-12 also was shifted down-field from its position of δ 147.8 to δ 157.6, again indicating a C=C–C=O system. Compound **6** was also oxidized to the ketone **26** in which the C-10 carbonyl signal was at δ 191.2 ppm, as was seen with **20**. The C-12 signal appeared at δ 157.3, compared to δ 142.8 in **6**. Thus, these oxidations to the keto compounds confirm the structures as assigned for **5** and **6**.

The presence of rotameric equilibrium in compound **5** as shown by the ^1H NMR spectrum in $CDCl_3$ taken at 20°C was referred to earlier. This behavior has been observed for taxchinins I and J by Tanaka *et al.* [13]. Interestingly, these three and taxayuntin F from *T. yunnanensis* [14] which also show this property, all have a hydroxyl at C-10 and a benzoate at C-9. However, three other compounds: **6** (shown here), the one reported by Chattopadhyay *et al.* [15] (same as taxacustin [14]), and taxayuntin E [14], all of which also contain a 10-hydroxyl but with an acetate at C-9, do not show this behavior. Likewise, the compound with the 9-hydroxy-10-benzoate structure gives a normal spectrum [7], as does the one in which 7, 9 and 10 carry hydroxyls [16]. It is generally known that members of this taxane subgroup with acyl groups at both 9 and 10 do not show this behavior.

The sensitivity of the ester functions to basic hydrolysis was studied briefly using **3**. The compound was relatively stable to alcoholic ammonia, but in alcoholic potassium carbonate, hydrolysis proceeded in 1 h to yield **6**, through the loss of the C-10 acetate. Continued action (2 h) gave a product in which the C-7, 9 and 10 esters were hydrolyzed, giving **21**, which was found to be identical by its spectral properties with the one described by Appendino *et al.* [16]. On standing for a longer time (12+ h), a pentaol (OHs generated at C-7, 9, 10 and 13) (**22**), as well as a hexaol (additional OH generated at C-4) (**23**) were formed. The pentaol (**22**) was described by Chen *et al.* [17] and **22** and **23** by Appendino *et al.* [16]. The rate of hydrolysis may be increased either by increasing the concentration of the carbonate or by using aqueous alcoholic carbonate. Acetylation of **22** yielded the tetraacetate **24**, whose NMR spectral data agreed with those given in [16].



21: $R_1 = Ac, R_2 = R_3 = R_4 = H, R_5 = Ac$

22: $R_1 = R_2 = R_3 = R_4 = H, R_5 = Ac$

23: $R_1 = R_2 = R_3 = R_4 = R_5 = H$

24: $R_1 = R_2 = R_3 = R_4 = R_5 = Ac$

25: $R_1 = R_2 = R_3 = R_4 = Ac, R_5 = H$

Fig. 3. Structures **21**–**25**.

Hydrolysis of compounds **2**, **3** and **5** was carried out using methanolic potassium hydroxide (0.5 N) for 6 h whereby each gave a single product, which was shown to be the same as **23**. Acetylation of the hexaol **23** gave a different tetraacetate **25**, with hydroxyls at 4 and 15 still remaining free. The proton NMR spectrum of **25** showed somewhat rounded peaks, but COSY spectrum indicated that the 4-hydroxyl remained free and that the oxetane function was still present. The spectral properties of **25** agreed with those described by Barboni *et al.* [18]. These hydrolytic experiments showed that the 2-benzoate was the most stable ester function in the 11(15 → 1)-abeotaxane system, perhaps because of the lack of assistance due to the 1-OH, usually present in the conventional taxane system. The oxetane ring also appears to be stable to mild alkaline treatment in this system.

The proton NMR spectra of compounds **11**–**14** suggested the presence of a 4/20 unsaturation (singlets for 20-H at 4.5 and 5.5 ppm, broad singlet for the 5-H, and ^{13}C signals for C-20 and C-4 at 115 and 145 ppm, respectively). The ^{13}C signals at 37–39 and 46–47 ppm which represent C-8 and C-15, respectively, suggested a 6-membered A-ring. Finally, a COSY spectrum showed coupling between H-13 and H-14 and between H-14 and H-1, thus indicating that C-1 carried not a hydroxyl but a proton. Thus compounds **11**–**14** all belong to the 11,4(20) taxadiene group, represented by taxinine J, with which **11** was found to be identical [19–21]. Compound **12** also contained a

cinnamate ester function at C-5 and was isolated earlier from the bark of *T. mairei* [21]. Compound **13** was identical with the taxane from the heartwood of *T. baccata* [22] and that of *T. mairei* [23]. Compound **14** was also found earlier in the heartwood of *T. baccata* [22]. This is the first report of the occurrence of **11**, **12**, **13** and **14** in the bark of *T. brevifolia*, although decinnamoyl taxinine J has been isolated from this source earlier by Kingston and coworkers [24]. The ^{13}C spectra for three of these have been unavailable and hence they are reported in the experimental section.

The ^1H NMR spectrum of compounds **9** and **10** also showed the presence of 4/20 unsaturation (singlets at 4.8 and 5.3 ppm, and a broad triplet at 5.3 ppm for H-5, at which site there is also an acetate. The three remaining protons attached to ester-linked carbons, each gave signals as doublets of doublets (H-10, ~6.1 ppm, H-2, ~5.4 ppm and H-14, 5.0 ppm). These assignments, verified by COSY spectra, showed that compounds **9** and **10** represent the select group of taxanes in which the ester side chain is at C-14, as seen in taiwanxan. Of these, compound **9**, called yunnanxane was isolated by Chen *et al.* [25] but no spectral data were available for comparison. The identity of **9** was established by comparing the spectral values with those given for 10-deacetyl yunnanxan [26]. The other member of this group, **10**, was found to be identical with the taxane described earlier [27, 28]. The occurrence of **9** and **10** in *T. brevifolia* is being reported here for the first time.

The ^1H and ^{13}C-NMR spectra of compounds **15**, shown in Table 3, gave indications for a 4/20 unsaturation (^1H signals for H-20 at 4.72 and 5.15 ppm, broad singlet at δ 5.24 for H-5, ^{13}C signals for C-20 and C-4 at δ 115 and 145, respectively). The presence of signals due to H-13 at δ 5.68 and that due to H-2 at δ 6.38, as well as the diagnostic signals for C-1 and C-15, suggested that a 5/7/6-membered ring system as in brevifoliol was present.

The general structural assignments were made on the basis of COSY and HETCOR spectra. In order to confirm the location of the benzoate groups, an HMBC spectrum was run. Long range interaction was observed between the ortho protons of one of the benzoates (δ 7.95) and the corresponding carbonyl signal at δ 166.9, which in turn, interacted with the H-2 (δ 6.38). Similar interaction was observed between the *o*-protons (δ 7.89) of the second benzoate, and the carbonyl (δ 164.2) and the H-10 (δ 6.73). Thus the location of the two benzoate groups was established at C-2 and C-10, thereby making **15** as a new member of this group.

In a review by Appendino [11], it was stated that all of the members of this group isolated so far, possessed the benzoate at C-10 and none at C-2. In this respect, **15** appears to be the first member with a benzoate at both C-2 and C-10. Unlike the rotameric equilibrium reported for some of the members of this group, we have not seen this behavior with crystalline brevifoliol, its 13-acetate or the new member, **15**, all of which gave normal spectra with sharp signals, when taken in $CDCl_3$ at room temperature.

Compound **16** was identified as baccatin VI by comparison with an authentic sample obtained earlier from *T. floridana* [30].

EXPERIMENTAL

^1H and ^{13}C NMR, COSY and the HETCOR spectra: Varian VXR-300 and Varian Gemini-300 spectrometers. Chemical shifts are reported in δ (ppm) using TMS as internal standard. FAB mass spectra: Finnigan Mat 950 Q spectrometer. IR spectra: Perkin–Elmer 1420 ratio-recording spectrophotometer. UV spectra: Perkin–Elmer Lambda 3B spectrophotometer.

Mps (uncorr): Fisher apparatus. TLC: silica gel 60 HF_{254} (E. Merck and Aldrich) with MeOH–Me_2CO–CH_2Cl_2 (1:4:15) or MeOH–CH_2Cl_2 (1:10) as solvents, visualization by UV (254 nm) and charring with dilute H_2SO_4 spay. Column chromatography: silica gel, 100–200 mesh (Aldrich).

Plant material

The bark of *T. brevifolia* was collected, dried and ground to 0.5″ mesh by Yew Wood Industries Company, 6928 North Interstate Avenue, Portland, OR 97217, from whom it was purchased during 1992.

Isolation of **2**, **3** *and* **4**

The reversed phase column procedure which gave the starting material was described in [6]. Briefly, C-18 bonded silica gel (12.5 kg) was loaded into a stainless steel column (6″ dia. and 6′ long) in 25% acetonitrile in water. The chloroform extract solids from the bark of *T. brevifolia* (obtained from 100 kg of the bark) was loaded onto the column, and the column eluted with the step gradient of acetonitrile in water (25–60%). After the elution of paclitaxel was completed (50% acetonitrile/water), subsequent fractions showed the presence of additional taxane components whose presence was detected by TLC (greenish blue when charred with dilute sulfuric acid spray). These were combined together and concentrated to dryness to yield 135 g of solid. Of this, a portion (30 g) was chromatographed on a silica column in CH_2Cl_2, using 0–10% Me_2CO in CH_2Cl_2 for elution.

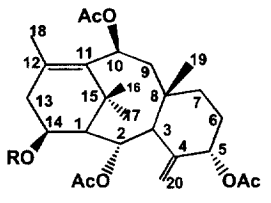
Compound **2** which was eluted with 5% Me_2CO–CH_2Cl_2, was obtained as a crystalline solid, mp 208–210°C (lit. 212–214°C [9]), yield, 0.9 g (4×10^{-3}% of bark). The spectral properties agreed with those reported in [8, 9].

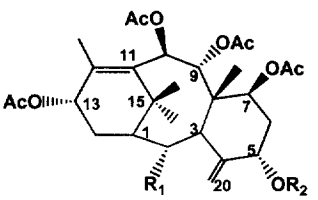
Compound **3** which was also eluted by the same solvent was obtained as colorless prisms, mp 203–205°C, yield, 2.25 g (0.01% of bark). The spectral data agreed with those given in [8].

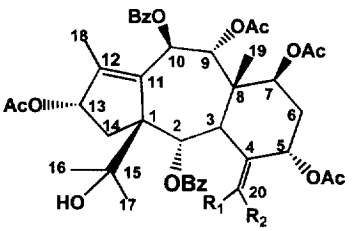
Compound **4** was also eluted by the same solvent,

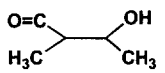
Table 3. ^{1}H and ^{13}C NMR spectra of compound **15**

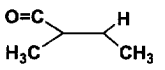
H	δ	C#	δ
1	—	1	69.1
2	6.38 (*d*, 9.0 Hz)	2	68.1
3	3.4 (*d*, 9.0 Hz)	2	43.3
4	—	4	139.3
5	5.24 (*br s*)	5	75.9
6α	1.86 (*m*)	6	34.9
6β	2.05 (*m*)	—	—
7	5.56 (*dd*, 10.8, 4.8 Hz)	7	68.8
8	—	8	45.1
9	6.12 (*d*, 10.8 Hz)	9	76.2
10	6.73 (*d*, 10.8 Hz)	10	69.0
11	—	11	135.5
12	—	12	148.6
13	5.68 (*t*, 6.9 Hz)	13	78.9
14α	2.11 (*m*)	14	37.9
14β	2.55 (*dd*, 14.1, 6.9 Hz)	—	—
15	—	15	75.5
16	1.18 (*s*)	16	27.8
17	1.19 (*s*)	17	25.8
18	2.10	18	12.0
19	1.16	19	13.4
20α	5.15 (*s*)	20	115.9
20β	4.72 (*s*)	—	—
Ac/Me	1.77, 2.08, 2.13, 2.19	Ac/Me	20.7, 21.3, 21.2, 21.3
Ac/CO	—	Ac/CO	169.5, 169.5, 169.8, 170.6
2-Bz-1″		2-Bz-1	128.9
2-Bz-2″,6″	7.95 (*d*, 7.8 Hz)	2-Bz-2″,6″	129.5
2-Bz-3″,5″	7.45 (*m*, 6.3 Hz)	2-Bz-3″,5″	128.6
2-Bz-4″	7.57 (*m*, 6.9 Hz)	2-Bz-4″	133.3
2-Bz-CO	—	2-Bz-CO	166.9
10-Bz-1‴	—	10-Bz-1‴	130.1
10-Bz-2‴,6‴	7.89 (*d*, 7.5 Hz)	10-Bz-2‴,6‴	129.7
10-Bz-3‴,5‴	7.45 (*t*, 6.3 Hz)	10-Bz-3‴,5‴	128.8
10-Bz-p	7.57 (*m*, 6.9 Hz)	10-Bz-p	133.4
10-Bz-CO	—	10-Bz-CO	164.2







9: R = 

10: R = 

	R_1	R_2
11:	OAc	Cinn.
12:	H	Cinn.
13:	H	Ac
14:	OAc	Ac

15: $R_1 = R_2 = H$

17: $R_1+R_2 = O$

Fig. 4. Structures **9**–**17**.

and was obtained as a colorless crystalline solid from ether, mp 210–212°C, yield, 0.75 g (3.4×10^{-3}% of bark).

Anal. Calc. for $C_{42}H_{48}O_{14} \cdot H_2O$: 63.47; H, 6.34. Found: C, 63.82; H, 6.38.

Compound **5** was eluted with 10% Me_2CO–CH_2Cl_2 and was obtained as a crystalline solid from Me_2CO-ligroin, mp 255°C, (dec). $[\alpha]_D$ (CH_2Cl_2), 21°, yield, 0.7 g (3.2×10^{-3}% of bark). IR (KBr, cm^{-1}): 3400, 3340, 1740–1710, 1595, 1575, 1485, 1470, 1450, 1370, 1310, 1270, 1240, 1170, 1110, 1065, 1020, 990, 930, 900, 700, 680. The ^1H spectrum run in $CDCl_3$ showed rounded and broad peaks. ^{13}C NMR ($CDCl_3$) also gave multiple signals for some protons. Anal. Calc. for $C_{45}H_{48}O_{13}$: 67.83; H, 6.07. Found: C, 68.07; H, 6.39.

Acetylation of **5** *to* **18**

A solution of **5** (0.1 g) in pyridine (0.5 ml) was treated with Ac_2O (2 ml) and the mixture let stand for 16 h. After dilution with water and filtration, the acetate was chromatographed (CH_2Cl_2-ligroin) and obtained as a white powder, yield, 0.1 g. ^1H NMR: (δ) 1.17, *s*, 3H, Me; 1.17, *s*, 3H, Me; 1.56, *s*, 3H, Me; 1.98, *s*, 3H, Me; 2.0, *m*, 2H, H-6 and H-14; 2.19, *s*, 3H, Me; 2.20, *s*, 3H, Me; 2.44, *dd*, 13.8, 6.9, Hz 1H, H-14; 2.56, *br s*, 1H, OH-15; 2.78, *m*, 1H, H-6; 3.21, *d*, 6.9 Hz, 1H, H-3; 4.21, *d*, 7.5 Hz, 1H, H-20, 4.56, *d*, 7.5 Hz, 1H, H-20; 5.74, *br t*, 1H, H-13; 5.89, *br t*, 1H, H-7; 6.49, *d*, 10.8 Hz, 1H, H-9, 6.53, *d*, 6.9 Hz, 1H, H-2; 6.61, *d*, 10.8 Hz, 1H, H-10; 6.86, 6.88, 6.91, 7.19, 7.21, 7.23, 7.37, 7.39, 7.41, 7.47, 7.50, 7.52, 7.60, 7.63, 7.65, 7.80, 7.92, 8.03 and 8.06, 15H, Ar-H. ^{13}C NMR: δ: 11.9, 13.2, 20.5, 21.1, 22.0, 25.1, 27.6, 27.6, 34.9, 36.8, 44.1, 44.5, 68.0, 68.4, 70.9, 74.5, 77.4, 78.7, 79.1, 84.6, 127.8, 128.0, 128.6, 129.6, 129.9, 130.5, 132.6, 133.5, 136.2, 147.2, 165.3, 166.4, 167.9, 169.0, 170.6. Anal. Calc. for $C_{47}H_{50}O_{14}$ 0.5 H_2O: C, 66.58; H, 6.06. Found: C, 66.55; H, 6.32.

Benzoylation of **5** *to* **19**

A solution of **5** (0.1 g) in pyridine (2 ml) was cooled and treated with benzoyl chloride (0.3 ml). After stirring for 20 h at room temperature, TLC showed reaction to be complete. Water was added and the solid filtered, chromatographed and treated as above, yield, 0.1 g. ^1H NMR: (δ) 1.21, *s*, 3H, Me; 1.90, *m*, 2H, H-6 and H-14; 2.01, *s*, 3H, Me; 2.03, *s*, Me; 2.19, *s*, 3H, Me; 2.22, *s*, 3H, Me; 2.48, *dd*, 14.1, 7.2 Hz, 1H, H-14; 2.68, *br s*, 1H, HO-15, 2.81, *m*, 1H, H-6; 3.28, *d*, 7.5 Hz, 1H, H-3; 4.23, *d*, 7.5 Hz, 1H, H-20; 5.02, *d*, 7.2 Hz, 1H, H-5; 5.74, *t*, 7.2 Hz, 1H, H-13; 5.97, *t*, 7.8 Hz, 1H, H-7; 6.61, *d*, 7.5 Hz, 2H, H-2; 6.64, *d*, 11.1 Hz, 1H, H-9; 6.66, *t*, 7.5 Hz, Ar-H; 6.87, *d*, 11.1 Hz, 1H, H-10; 7.01, *t*, 7.5 Hz, Ar-H; 7.12, 7.15, 7.17, 7.19, 7.21, 7.28, 7.31, 7.32, 7.35, 7.48, 7.50, 7.53, 7.55, 7.57, 7.61, 7.63, 7.66, 7.93 (*d*, 7.5 Hz), 8.06, *d*, 7.2 Hz, Ar-H. ^{13}C NMR: δ 13.2, 21.2, 22.0, 22.0, 25.6, 27.8, 35.0, 36.9, 44.2, 44.6, 53.4, 68.2, 68.6, 71.0, 75.8, 78.8, 79.1, 84.7, 127.3, 128.0, 128.2, 128.6, 128.9, 129.1, 129.5, 129.7, 130.0, 130.6, 132.2, 132.6, 132.9, 133.5, 136.2, 147.8, 164.2, 165.3, 165.9, 166.6, 169.0, 170.7. Anal. Calc. for $C_{52}H_{52}O_{14}$ 0.5 H_2O: C, 68.64; H, 5.87. Found: C, 68.48; H, 6.06.

Oxidation of **5** *to* **20**

Compound **5** (0.1 g) in Me_2CO (3 ml) was treated with Jones reagent, and the mixture stirred until TLC showed the reaction to be complete. Water was added, the mixture extracted with CH_2Cl_2, the extract concentrated to dryness and the product chromatographed on silica (CH_2Cl_2-ligroin) to give a crystalline solid, mp 240°C (dec), yield, 0.06 g. ^1H NMR: (δ) 1.19, *s*, 3H, Me; 1.23, *s*, 3H, Me; 1.85–2.05, *m*, 1H, H-14; 2.15, *s*, 3H, Me; 2.15, *s*, 6H, 2 × Me; 2.20, *s*, 3H, Me; 2.54, *dd*, 14.4, 7.2 Hz, 1H, H-14; 2.91, *m*, 1H, H-6; 3.32, *d*, 7.2 Hz, 1H, H-3; 4.26, *d*, 7.5, Hz 1H, H-20β; 4.99, *d*, 5.7 Hz, 1H, H-5; 5.49, *dd*, 8.7, 5.7 Hz, 1H, H-7; 5.83, *t*, 7.5 Hz 1H, H-13; 6.51, *s*, 1H, H-9; 6.59, *d*, 7.5 Hz, 1H, H-2; 6.85, *t*, 7.8 Hz, 2H; 7.27, *t*, 7.8 Hz; 1H; 7.40, *t*, 7.2 Hz, 2H; 7.48, 7.51, 7.53, 7.55, 7.62, 7.64, 7.70, *d*, 7.2, 2H; 8.05 (*d*, 8.7 Hz), 8.08 (*d*, 8.4 Hz), Ar-H. ^{13}C NMR: 14.0, 21.0, 21.9, 25.5, 27.6, 34.7, 37.2, 44.1, 45.0, 65.9, 69.2, 69.3, 71.2, 74.6, 76.5, 78.7, 79.0, 84.2, 84.9, 127.8, 128.3, 128.7, 128.9, 129.7, 129.8, 129.9, 130.8, 132.8, 132.9, 133.7, 137.6, 157.6, 165.1, 165.9, 167.1, 168.9, 170.5, 191.8. Anal. Calc. for $C_{45}H_{46}O_{13}$, C, 67.96; H, 5.83. Found: C, 67.63; H, 5.70.

Isolation of **6**, **8**, *and* **9**

The mother liquors from the crystallization of **5**, together with the eluates of the initial column with 10–20% Me_2CO in CH_2Cl_2 were combined and chromatographed using Me_2CO/CH_2Cl_2 mixtures. The sequential elution gave compounds **9**, **5**, **8** and **6** in that order.

Compound **6** was obtained as a white powder, yield, 0.25 g (1.1×10^{-3}% of bark), IR (KBr, cm^{-1}): 3560, 1735, 1720, 1450, 1365, 1270, 1235, 1170, 1110, 1065, 1025, 980, 935, 705. (See Table 1 for the NMR spectra.) The compound was characterized as its monoacetate, which was a crystalline solid, mp 203–205°C, identical with **3**. Anal. Calc. for $C_{42}H_{48}O_{14}$: C, 64.94; H, 6.23. Found: C, 64.95; H, 6.47.

Oxidation of **6** *to* **26**

The procedure described under **20** was repeated using **6** (0.1 g) and the product **26** was obtained as a white powder, yield, 0.4 g. ^1H NMR (δ) 1.12, *s*, 3H, Me; 1.18, *s*, Me; 1.56, *s*, Me; 2.05, *s*, Me; 2.18, *s*, Me; 2.19, *s*, Me; 2.20, *s*, Me; 2.48, *ss*, 14.1, 7.2 Hz, H-14; 2.77, *dt*, 15.9, 8.1 Hz, H-6; 3.23, *d*, 7.5 Hz, H-3; 4.22, *d*, 7.5 Hz, H-20; 4.55, *d*, 7.5, Hz, H-20; 4.99, *d*, 6.9 Hz, H-5; 5.41, *dd*, 9.0, 8.4 Hz, H-7; 5.81, *t*, 7.2 Hz, H-13; 6.19, *s*, H-9; 6.28, *d*, 7.8 Hz, H-2; 7.44–7.67, *m*, Ar-H;

8.03, *d*, 7.5 Hz, Ar-H; 8.16 *d*, 7.2 Hz, Ar-H. ^{13}C NMR: 13.5, 13.9, 20.0, 21.0, 21.9, 25.5, 27.5, 34.6, 37.0, 44.4, 44.7, 65.9, 68.9, 70.6, 74.4, 76.3, 78.7, 78.9, 84.0, 84.7, 128.3, 128.7, 129.6, 129.8, 130.6, 132.9, 133.7, 137.7, 157.3, 164.6, 165.9, 168.9, 170.5, 170.9, 191.2. Anal. Calc. for $C_{40}H_{44}O_{13}$. H_2O: C, 63.99; H, 6.18. Found: C, 63.75; H, 6.20.

Compound **8** was obtained as a crystalline solid, yield, 0.45 g (2×10^{-3}% of bark), mp 164–166°C. $[\alpha]_D$, −77.5°, IR: 3580, 3560, 1735, 1715, 1595, 1575, 1445, 1365, 1310, 1250, 1175, 1105, 1090, 1065, 1020, 985, 930, 910, 860, 840, 705. (See Table 1 for the NMR spectra. Anal. Calc. for $C_{45}H_{48}O_{13}$, C, 67.83; H, 6.07. Found: C, 67.62; 6.29.

Compound **9** was obtained as a white powder, yield, 0.1 g (4.5×10^{-4}% of bark). The spectral data indicated that it was identical with yunnanxane [25].

Isolation of **7** *and* **10–16**

The mother liquors from **2**, **3** and **4** and the fractions from the initial column collected before these, were combined, concentrated and chromatographed on a silica column in EtOAc-ligroin mixtures, starting with a 1:4 ratio. Elution gave successively, **10**, **12**, **13**, a mixture of **7**, **11** and **15**, followed by **14** and **16**.

Compound **10** was obtained as white powder, yield, 0.02 g (1×10^{-4}% of bark). Its spectral data showed it to be identical to the taxane reported in [27, 28].

Compound **12** was obtained as a crystalline solid, mp 170–171°C (lit. 171–172°C [29]), yield, 0.4 g (1.8×10^{-3}% of bark). The physical and spectral properties agreed with those described in [21]. ^{13}C spectrum: (δ) 13.2, 15.3, 20.8, 21.0, 21.5, 27.2, 27.3, 31.2, 31.9, 34.6, 37.5, 39.4, 40.2, 46.3, 70.0, 70.6, 71.7, 74.8, 76.7, 116.0, 118.4, 128.1, 129.0, 130.6, 134.1, 135.0, 137.2, 145.7, 146.3, 166.1, 169.3, 169.9, 170.2 and 170.7.

Compound **13** was obtained as a colorless crystalline solid, mp 201–203°C (lit. 205–207°C [29]), yield, 0.06 g (2.7×10^{-4}% of bark). Its physical and spectral data were identical with those given in [22, 23].

Compound **14** was obtained as a colorless crystalline solid, mp 197–199°C (lit. 197°C, [29]), yield, 0.03 g (1.1×10^{-4}% of bark). Its physical and spectral properties agreed with those given in [22]. ^{13}C spectrum: (δ) 13.,8, 15.9, 20.8, 21.0, 21.4, 21.5, 21.5, 27.4, 28.0, 31.6, 34.9, 37.6, 43.0, 47.2, 49.0, 69.9, 69.9, 70.4, 70.7, 71.5, 75.9, 119.1, 133.0, 137.3, 140.1, 169.1, 169.2, 169.4, 169.8, 169.8, and 170.4.

Compound **16** was obtained as a colorless crystalline solid, mp 250–252°C, yield, 0.15 g (6.8×10^{4}% of bark). Its physical and spectral properties showed that it was identical with baccatin VI [30].

The mixture of **7**, **11** and **15** was taken up in MeOH and let stand for 24 h, whereby a crystalline solid was obtained. This was filtered, washed and recrystallized from MeOH to obtain **11** as a colorless needles, mp 248–249° (lit. 248–249° [29]), yield, 0.065 g (2.9×10^{-4}% of bark). Its physical and spectral properties agreed with those given in [19–21]. ^{13}C spectrum: (δ) 13.6, 15.8, 20.7, 21.0, 21.3, 21.4, 27.1, 28.3, 31.6, 35.2, 37.6, 42.7, 47.1, 48.6, 69.8, 70.4, 70.8, 71.6, 75.8, 76.0, 118.3, 118.9, 128.1, 129.0, 130.6, 133.6, 134.1, 137.0, 140.3, 146.0, 166.1, 169.2, 169.3, 169.7, 169.8 and 170.6.

The mixture of **7** and **15** (0.5 g) in CH_2Cl_2 (20 ml) was cooled in a dry ice/acetone bath and ozone was bubbled through the solution for 10–15 min. The mixture was removed from the bath, treated with dimethyl sulfide (0.5 ml) and let stand for 2 h. After concentration to dryness, the residue was purified by preparative TLC using 4:1 ligroin:Me_2CO. The faster band was eluted (CH_2Cl_2–MeOH (9:1)) and concentrated to dryness to obtain **7** as a crystalline solid, mp 234–236°C; yield, 0.025 g (1.1×10^{-4}% of bark). IR (KBr, cm^{-1}): 3560, 3450, 1735, 1720, 1450, 1365, 1265, 1235, 1225, 1170, 1110, 1065, 1020, 980, 705. Anal. Calc. for $C_{47}H_{50}O_{14}$. $2H_2O$: C, 64.52; H, 6.22. Found: C, 64.85; H, 6.14.

The slower band was processed likewise and **17** (the ketone product from **15**) was obtained as a crystalline solid, mp 245°C (*d*), yield, 0.2 g, IR (KBr, cm^{-1}): 3480, 1755, 1720, 1440, 1370, 1215, 1170, 1095, 1060, 1020, 700. ^{1}H NMR: (δ) 1.13, 3H, Me; 1.22, *br s*, 6H, 2 × Me; 2.07. *s*, 3H, Ac; 2.09, *s*, 3H, Ac; 2.14, *s*, 3H, Ac; 1.95–2.20, *m*, 2H, 6-Hα and 6-Hβ; 1.95–2.15, *m*, 1H, H-14α; 2.48, *dd*, 14.1, 6.9 Hz, 1H, H-14β; 3.69, *d*, 8.7 Hz, 1H, H-3; 4.56, *br s*, 1H, H-5; 5.67, *t*, 7.2 Hz, 1H, H-13; 5.79, *dd*, H-7; 6.22, *d*, 10.2 Hz, H-9; 6.33, *d*, 7.8 Hz, 2H, H-2; 6.72, *d*, 10.2 Hz, 1H, H-10; 7.42, 7.44, 7.47, 7.52, 7.57, 7.87, 7.90, 7.97 and 7.99, *m*, Bz-ArH. ^{13}C NMR: 12.1, 13.8, 20.6, 21.2, 21.2, 23.5, 25.5, 27.4, 33.7, 37.6, 37.6, 47.1, 47.6, 65.6, 67.3, 67.8, 68.4, 74.5, 75.3, 75.7, 78.8, 128.4, 128.8, 129.6, 129.7, 132.8, 133.6, 135.9, 137.7, 148.7, 165.7, 3 × 169.3, 170.4, 199.8. Anal. Calc. for $C_{41}H_{46}O_{14}$: C, 64.55; H, 6.08. Found: C, 64.17; H, 6.20.

Direct isolation of **15**

The mixture of **7** and **15** was chromatographed on a silica column using 1% acetone in benzene, which gave separation of the two components. Fractions containing **15** were concentrated and the product was obtained as a crystalline solid, mp 200–203°C, $[\alpha]_D$, −21.5°, yield, 0.045 g (2×10^{-4} of bark). IR, (KBr, cm^{-1}): 3540, 1730, 1440, 1360, 1255, 1230, 1170, 1110, 1060, 1020, 705. Anal. Calc. for C, 66.3; H, 6.36. Found: 66.47; H, 6.57.

Alkaline hydrolysis of **3**

(a) A solution of **3** (0.4 g) in MeOH (20 ml) was treated with K_2CO_3 (100 mg) and the mixture stirred at room temperature for 1 h. It was extracted with CH_2Cl_2 and the extract concentrated and chromatographed on silica using the same solvent with 5–10% acetone. Besides the unreacted **3**, the major

product was recovered and shown to be identical with **6**.

(b) The above reaction was continued for 2–3 h, at which time the starting material was consumed. The products were recovered and purified by chromatography as before. The major product was **21**, which was found to be identical with a tetraol described in [16]. The second and minor band was isolated and shown to be the same as the pentaol described in [16, 17]. Acetylation of an aliquot gave the acetate **24**, the spectral data of which agreed with the corresponding compound described in [16].

(c) To a solution of **3** (0.2 g) in MeOH (15 ml) was added K_2CO_3 (10%, 5 ml). After 30 min when the starting material was absent the mixture was neutralized, extracted with EtOAc, and the extract concentrated to dryness. Chromatography in CH_2Cl_2/Me_2CO gave the pentaol **22**, the ^1H NMR spectral data of which agreed with those given in [16].

(d) A solution of **2**, **3** or **5** (0.2 g) in MeOH was treated with methanolic KOH (0.5 N, 5 ml) and the mixture stirred at room temperature for 4–6 h, at which time the starting material was absent, and one major compound could be seen in TLC. After partial neutralization, the mixture was extracted with EtOAc and the product chromatographed on silica using CH_2Cl_2/Me_2CO mixtures. The product **23** was recovered as a white powder, yield, 0.08 g. Its spectral data showed identity with the hexaol in [16]. Acetylation of an aliquot gave the tetraacetate **25**, whose spectral properties agreed with those described in [18].

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