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ANTITUMOR AGENTS, 135. ¹ STRUCTURE AND STEREOCHEMISTRY
OF POLACANDRIN, A NEW CYTOTOXIC TRITERPENE
FROM *POLANISIA DODECANDRA*

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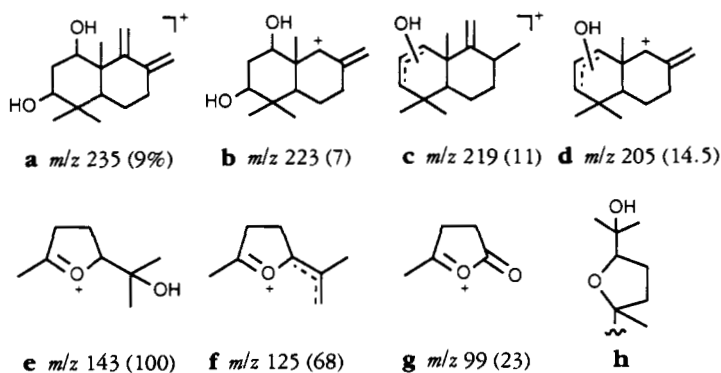
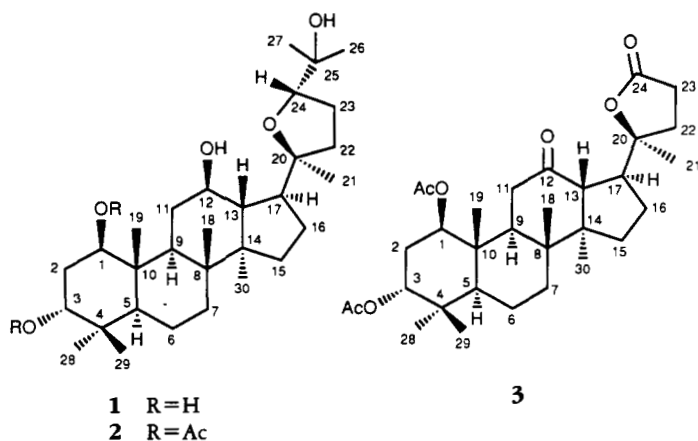
ABSTRACT.—A new dammarane triterpene, polacandrin [**1**], has been isolated from *Polanisia dodecandra*. The structure of **1** was established as $1\beta,3\alpha,12\beta,25$ -tetrahydroxy-20(*S*),24(*S*)-epoxydammarane by chemical and spectroscopic methods, which included the concerted application of a number of 2D nmr techniques that involved ^1H - ^1H COSY, HETCOR, NOESY, and long-range HETCOR. Single-crystal X-ray analysis of polacandrin pentahydrate verified the complete structure and stereochemistry. Polacandrin showed potent cytotoxicities against KB ($\text{ED}_{50} = 0.60 \mu\text{g/ml}$), P-388 ($\text{ED}_{50} = 0.90 \mu\text{g/ml}$), and RPMI-7951 ($\text{ED}_{50} = 0.62 \mu\text{g/ml}$) tumor cells.

Polanisia dodecandra (L.) DC. (Capparidaceae), Clammy-weed, a branched glandular-viscid herb with a rank odor that grows in sandy places, is found from Montana to Wisconsin and Quebec to Mexico (1). *P. dodecandra* is a hitherto phytochemically and biologically uninvestigated species. In the course of our continuing search for novel cytotoxic antitumor agents that are active against slowly growing solid tumors, especially those compounds derived from previously uninvestigated plant sources, the MeOH extract of the whole plant of *P. dodecandra* was found to exhibit significant in vitro cytotoxicity against KB, A-549, HCT-8, RPMI-7951, and P-388 tumor cells. Bioassay-directed fractionation of the active extract has led to the isolation and characterization of a new cytotoxic principle, polacandrin [**1**], a dammarane-type triterpene. We report herein on the isolation and structural elucidation of **1**.

RESULTS AND DISCUSSION

The MeOH extract of the whole plant of *P. dodecandra* was concentrated and partitioned by *n*-hexane, CHCl_3 , *n*-BuOH, and H_2O . Guided by the assay in RPMI cells, the active principles were concentrated in the CHCl_3 and *n*-BuOH extracts. Si gel

¹For part 134, see H.K. Wang, J.X. Xie, J.J. Chang, K.M. Hwang, S.Y. Liu, L.M. Ballas, J.B. Jiang, and K.H. Lee, *J. Med. Chem.*, **35**, 2717 (1992).



chromatography of the latter followed by flash chromatography on reversed-phase Si gel afforded the active principle, polacandrins [**1**], in 0.008% yield.

Compound **1** was isolated as colorless prisms, mp 234–238°, and was positive to a Liebermann-Burchard reaction, giving a purple color. The FAB/MS of **1** gave an $[M + 1]^+$ ion at m/z 493.3900 ($C_{30}H_{53}O_5$, calcd 493.3893), whereas the EIMS obtained at 70 eV failed to show the true molecular ion, but gave an apparent $[M - H_2O]^+$ ion at m/z 474.3666 ($C_{30}H_{50}O_4$, calcd 474.3709) and a base peak at m/z 143 ($C_8H_{15}O_2$). The IR spectrum indicated the presence of hydroxyl (3400 and 3340 cm^{-1}) and ether (1083 and 1030 cm^{-1}) groups. Signals for eight tertiary methyl groups were present in the $^1\text{H-NMR}$ spectrum (Table 1). The $^{13}\text{C-NMR}$ spectrum (Table 2) and DEPT and HETCOR experiments indicated that **1** contained 30 carbon atoms and 48 carbon-bonded hydrogen atoms. The nature of the carbon centers was revealed by DEPT as eight methyls, eight methylenes, four simple methines, four oxygenated methines, four quaternary carbons, and two oxygenated quaternary carbons. These data were consistent with the HRMS empirical formula and indicated that **1** was a triterpene. Prominent peaks at m/z 143 and 349 in the EIMS of **1** were characteristic of tetracyclic triterpenes (2,3). Comparison of the carbon resonances with those of related dammarane-type triterpenes (2) confirmed that **1** possessed the same basic skeleton.

The $^1\text{H-NMR}$ spectrum of **1** showed that a number of protons were deshielded. A triplet at δ 3.46 ($J = 3.0\text{ Hz}$) was typical for the equatorial oxymethine proton at C-3 of triterpenes (4). Support for the α -hydroxyl stereochemistry at C-3 was derived from the $^{13}\text{C-NMR}$ spectral data. A downfield doublet at δ 76.5 was ascribed to C-3 based

TABLE 1. ^1H -nmr Spectral Data for **1**, **2**, and **3**.

Proton	Compound		
	1	2	3
H-1 . . .	3.82, dd, $J = 5.0, 10.5$ Hz	4.86, dd, $J = 5.5, 10.5$ Hz	4.80, dd, $J = 5.0, 11.0$ Hz
H-2 . . .	1.82, m	1.83, m	α 1.90, m; β 1.82, m
H-3 . . .	3.46, t, $J = 3.0$ Hz	4.75, t, $J = 3.0$ Hz	4.75, t, $J = 3.0$ Hz
H-4 . . .	—	—	—
H-5 . . .	1.30, m	1.25, m	1.33, dd, $J = 2.5, 10.0$ Hz
H-6 . . .	1.48, m	1.50, m	1.58, m
H-7 . . .	α 1.50, m β 1.27, m	α 1.50, m β 1.27, m	α 1.58, m β 1.40 dt, $J = 3.0, 12.5$ Hz
H-8 . . .	—	—	—
H-9 . . .	1.77, m	1.77, m	2.01, dd, $J = 5.0, 13.5$ Hz
H-10 . . .	—	—	—
H-11 . . .	α 2.70, dt, $J = 4.0, 13.0$ Hz β 1.28, m	α 1.77, m β 1.28, m	α 2.34, t, $J = 13.5$ Hz β 2.15, dd, $J = 5.0, 13.5$ Hz
H-12 . . .	3.58, td, $J = 5.0, 10.0$ Hz	3.50, td, $J = 5.0, 10.0$ Hz	—
OH . . .	6.04, s	5.46, s	—
H-13 . . .	1.66, t, $J = 10.0$ Hz	1.65, t, $J = 10.0$ Hz	2.86, d, $J = 10.0$ Hz
H-14 . . .	—	—	—
H-15 . . .	α 1.50, m β 1.08, dd, $J = 8.0, 11.5$ Hz	α 1.50, m β 1.08, m	α 1.74, m β 1.22, m
H-16 . . .	α 1.95, m β 1.28, m	α 1.95, m β 1.27, m	α 1.87, m β 1.58, m
H-17 . . .	2.23, td, $J = 4.0, 10.0$ Hz	2.23, td, $J = 5.0, 10.0$ Hz	2.60, m
H-18 . . .	1.01, s	1.07, s	1.22, s
H-19 . . .	0.95, s	1.02, s	1.12, s
H-20 . . .	—	—	—
H-21 . . .	1.25, s	1.26, s	1.25, s
H _a -22 . . .	1.95, m	2.01, m	2.18, m
H _b -22 . . .	1.76, m	1.75, m	1.94, m
H-23 . . .	1.89, m	1.82, m	2.61, m
H-24 . . .	3.89, dd, $J = 5.0, 5.5$ Hz	3.88, dd, $J = 5.5, 6.0$ Hz	—
H-25 . . .	—	—	—
H-26 . . .	1.19, s	1.23, s	—
H-27 . . .	1.13, s	1.09, s	—
H-28 . . .	0.90, s	0.92, s	0.95, s
H-29 . . .	0.82, s	0.84, s	0.86, s
H-30 . . .	0.93, s	0.93, s	0.79, s
Ac	—	2.03, s; 2.12, s	2.01, s; 2.11, s

upon the fact that the axial hydroxyl-bearing resonance C-3 in this type of compound is generally found at ca. δ 75 whereas for the corresponding equatorial substituent it lies at ca. δ 80 (2). The observation of an nOe between the signal at δ 3.46 and the two C-4 methyl signals at δ 0.82 and 0.90 in the NOESY spectrum of **1**, coupled with its ^{13}C -nmr spectrum, further confirmed the α configuration of the hydroxyl at C-3.

Prominent fragment ions appearing in the mass spectrum of **1** at m/z 235 (**a**), 223 (**b**), 219 (**c**), and 205 (**d**) are derived from rings A and B of pentacyclic or tetracyclic triterpenoids (5,6). Consequently, these data suggested that the two hydroxyl groups were situated on either ring A and/or ring B. In the ^1H -nmr spectrum of **1**, a signal due to a proton geminal to a secondary hydroxyl group occurred as a doublet of doublets at δ 3.82, which is typical of the X part of an ABX system. The large coupling constants ($J = 5.0$ and 10.5 Hz) defined the hydroxyl disposition as equatorial and flanked by quaternary carbon and methylene carbon centers. This evidence suggested that the hy-

TABLE 2. ^{13}C -nmr Spectral Data for **1**, **2**, and **3**.

Carbon	Compound		
	1	2	3
C-1	74.7, d	78.0, d	78.2, d
C-2	36.4, t	29.4, t	28.9, t
C-3	76.5, d	78.8, d	77.8, d
C-4	37.3, s	36.6, s	36.6, s
C-5	48.3, d	50.0, d	49.8, d
C-6	18.4, t	18.1, t	18.1, t
C-7	34.6, t	34.4, t	33.7, t
C-8	40.6, s	40.6, s	41.1, s
C-9	50.9, d	50.3, d	53.7, d
C-10	43.4, s	42.3, s	42.5, s
C-11	34.3, t	33.5, t	42.1, t
C-12	70.9, d	70.4, d	209.1, s
C-13	48.2, d	48.1, d	56.3, d
C-14	52.0, s	51.9, s	55.2, s
C-15	32.4, t	32.2, t	31.6, t
C-16	28.3, t	28.4, t	24.2, t
C-17	49.0, d	49.4, d	42.9, d
C-18	15.7, s	15.8, q	16.3, q
C-19	12.3, q	13.1, q	13.1, q
C-20	87.1, s	87.1, s	88.8, s
C-21	28.8, q	28.9, q	24.9, q
C-22	31.7, t	31.6, t	31.6, t
C-23	25.5, t	25.1, t	28.9, t
C-24	87.5, d	87.3, d	177.0, s
C-25	70.6, s	70.2, s	—
C-26	27.3, q	27.4, q	—
C-27	23.8, q	24.2, q	—
C-28	28.0, q	28.0, q	27.4, q
C-29	21.9, q	22.3, q	21.4, q
C-30	17.9, q	17.5, q	16.5, q
Ac		21.6, q; 21.1, q; 170.6, s; 170.7, s	21.8, q; 21.2, q 170.0, s; 170.5, s

droxyl group was β -oriented either at C-1 or C-7. A strong coupling response between signals at δ 3.82 and 1.82 in the ^1H - ^1H COSY spectrum of **1** as well as a strong cross peak between those at δ 3.46 (H-3) and 1.82 effectively established the nature of rings A and B in which the location of the hydroxyl group was unequivocally assigned to C-1 rather than C-7. Furthermore, a comparison of the ^{13}C -nmr spectrum of **1** with those of related dammarane-type triterpenes (**2**) indicated that, although other carbon resonances remained almost unchanged, the C-10 and C-2 carbon signals occurred somewhat downfield due to the fact that C-1 was deshielded. Assignment of hydroxyls at C-1 and C-3 was substantiated by a long-range HETCOR experiment, which revealed long-range coupling through two and three bonds. Thus, the Me-28 protons (δ 0.90) showed three-bond correlation to the signals at δ 76.5 (C-3), 48.3 (C-5), and 21.9 (C-29) as well as two-bond correlation to that at δ 37.3 (C-4), while H-3 (δ 3.46) was correlated with the signals at δ 37.3 (C-4, 2 bond), 21.9 (C-29, 3-bond), and 48.3 (C-5, 3-bond). The Me-19 protons (δ 0.95) showed two-bond correlation to the signal at δ 43.4 (C-10) and three-bond coupling to those at δ 74.7 (C-1), 50.9 (C-9), and 48.3 (C-5). These data were consistent with the presence of hydroxyls at C-1 and C-3.

In the ^1H -nmr spectrum of **1**, a sharp singlet at δ 6.04 was assigned to the 12-OH proton based upon the fact that it was similar to that for 12 β -hydroxy-20,24-epoxy-25-hydroxydammarane-type derivatives in which it was proposed that the hydroxy group was involved in an intramolecular hydrogen bond with the furan ring oxygen atom (7). A triplet of doublets at δ 3.58, showing two large and one small coupling ($J = 5.0$ and 10.0 Hz), was indicative of an axial oxymethine proton in the $\text{CH}_2\text{CH}(\text{OH})\text{CH}$ partial structure; it was assigned to H-12. One further feature of the spectrum was the occurrence of two separate proton resonances at δ 2.70 [doublet of triplets ($J = 4.0$ and 13.0 Hz)] and δ 2.23 [triplet of doublets ($J = 4.0$ and 10.0 Hz)]. The former signal can be assigned to H-11 α and the latter to H-17 on the basis of HETCOR and ^1H - ^1H COSY spectra. According to their coupling patterns, these two protons must be axially disposed and coupled to three protons. In the ^1H - ^1H COSY spectrum, the signal at δ 2.70 showed strong coupling responses to H-9 (δ 1.77), H-11 β (δ 1.28), and H-12 (δ 3.58), which in turn was correlated to H₂-11 and H-13 (δ 1.66). The proton signal at δ 2.23 was coupled to H-13 and H₂-16, while H₂-16 showed correlation to H-17 and H₂-15. This spin system, revealed by the ^1H - ^1H COSY spectrum as described above, further confirmed that **1** was a dammarane-type triterpene.

Further confirmation for the location of the hydroxy groups at C-1, C-3, and C-12 was derived in the following manner. Acetylation of **1** yielded a diacetate **2**, the hrfabms spectrum of which gave an $[\text{M} + 1]^+$ ion at m/z 577.4082. In addition to containing the expected two acetoxy group signals (δ 2.03 and 2.12), the ^1H -nmr spectrum of **2** revealed that the signals for H-1 and H-3 [δ 4.86 (dd, $J = 5.5, 6.0$ Hz) and δ 4.75 (t, $J = 3.0$ Hz), respectively] were shifted downfield from the corresponding signals for **1**, whereas the H-12 and the 21-OH proton resonances were essentially unchanged. Subsequent oxidation of **2** with Jones' reagent gave a keto derivative **3**. That **3** contained a six-membered ring carbonyl group was indicated by the presence of a signal at δ 209.1 in the ^{13}C -nmr spectrum, while the ^1H -nmr spectrum lacked a signal for H-12. Moreover, a methine signal at δ 2.86 (d, $J = 10.0$ Hz), assignable to H-13 and shown to be coupled to H-17 on the basis of the ^1H - ^1H COSY spectrum, was shifted downfield, consistent with the presence of the C-12 carbonyl group.

In the ^{13}C -nmr spectrum of **1**, singlets situated at δ 70.6 and 87.1 are typical of C-25 and C-20 resonances for all 20,24-epoxy-25-hydroxy dammaranes (2). The ms of **1** contained a base peak at m/z 143 and a prominent peak at m/z 125, which are attributable to fragments **e** and **f**, respectively, and are characteristic of partial structure **h** of all 20,24-epoxy-25-hydroxyl dammarane-type triterpenes (3). A doublet of doublets at δ 3.89 in the ^1H -nmr spectrum was also typical of H-24 in the tetrahydrofuran side chain (8). This evidence revealed that the side chain in **1** is similar to that of related compounds such as betulafolientriol oxide II and its related derivatives, a fact which was further corroborated by oxidation of the diacetate **2** with Jones' reagent to yield a trisnor- γ -lactone **3**. Compound **3** analyzed for $\text{C}_{31}\text{H}_{46}\text{O}_7$ and gave a fragment peak at m/z 99 (23%), indicative of the appearance of ion **g**. The ^{13}C -nmr spectrum of **3** also revealed the presence of an ester carbonyl in addition to a six-membered ring carbonyl, while it lacked resonances for C-25, C-26, and C-27. The ^1H -nmr spectrum of **3** showed that only six of the eight methyl groups in **2** remained and H-24 was lost. The five-membered γ -lactone ring was also revealed by the ir spectrum (1770 cm^{-1}).

The 20*S*,24*S* stereochemistry in **1** was ascertained from 2D ^1H nOe (NOESY) spectral evidence (Figure 1). Since the tetrahydrofuran ring is not free to rotate due to intramolecular O . . HO hydrogen bonding to the 12-OH group, the NOESY spectrum showed strong nOe response between the protons at C-21 (δ 1.13) and H-17, thereby establishing that Me-21 and H-17 were similarly disposed and in close proximity. Moreover, strong nOe responses between Me-21 and the alkyl group at C-24 indi-

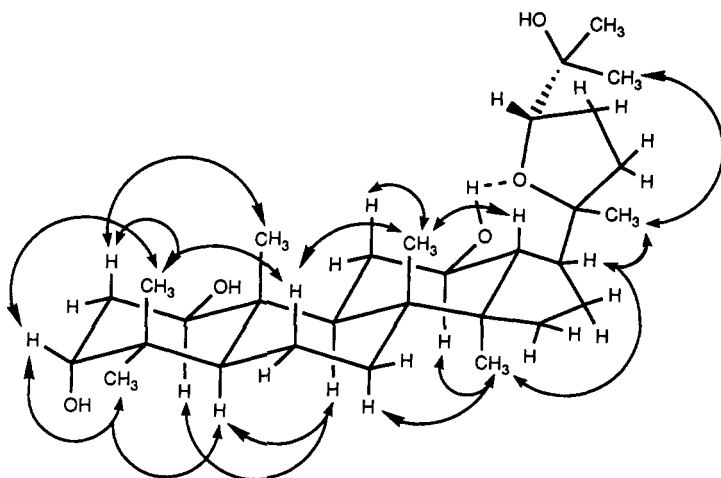


FIGURE 1. The nOe responses of 1.

cated that these groups must be *cis* on the tetrahydrofuran ring. Furthermore, signals for the non-equivalent methyl groups at C-25 (δ 1.19 and 1.13) are in accord with those reported for 20*S* compounds in contrast to the situation with 20*R* compounds for which the C-25 methyl group resonances either are identical or lie within 0.03 ppm of each other (8). The ¹³C-nmr resonance for C-24 in 1 at δ 87.5 is also in agreement with the published value for 24*S* compounds (2).

The ¹H-¹H COSY and HETCOR were used for assignment of all proton resonances as shown in Table 1. The long-range HETCOR spectrum was also used for assignment of all carbon resonances listed in Table 2; Figure 2 summarizes the observed long-range correlations.

From the foregoing evidence, the structure of 1 was assigned as 1 β ,3 α ,12 β ,25-tetrahydroxy-20(*S*),24(*S*)-epoxydammarane. An X-ray crystallographic analysis of the pentahydrate unequivocally established the complete structure and relative stereochemistry. The crystal structure was solved by direct methods. Non-hydrogen

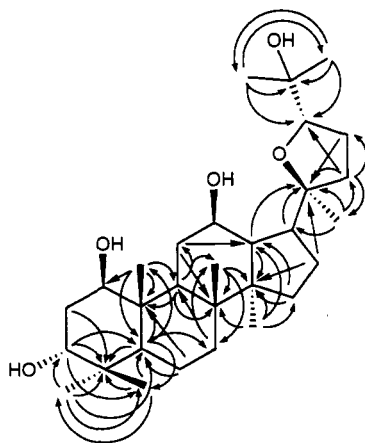


FIGURE 2. The long-range HETCOR responses of 1.

atom fractional coordinates are listed in Table 3.² A view of the solid-state conformation, with the atom numbering scheme indicated, is provided in Figure 3. Bond lengths are in accord with expectations (9). Endocyclic torsion angles (ω_{ij} , $\sigma \pm 0.04\text{--}0.05^\circ$) about the bonds between atoms i and j follow: $\omega_{1,2} -55.7$, $\omega_{2,3} 56.4$, $\omega_{3,4} -52.4$, $\omega_{4,5} 52.7$, $\omega_{5,10} -49.8$, $\omega_{10,1} 49.2^\circ$ in ring A; $\omega_{5,6} -65.2$, $\omega_{6,7} 57.7$, $\omega_{7,8} -47.3$, $\omega_{8,9} 47.8$, $\omega_{9,10} -53.9$, $\omega_{10,5} 60.6^\circ$ in ring B; $\omega_{8,9} -57.7$, $\omega_{9,11} 57.7$, $\omega_{11,12} -57.1$, $\omega_{12,13} 57.4$, $\omega_{13,14} -61.0$, $\omega_{14,8} 60.7^\circ$ in ring C; $\omega_{13,14} 45.1$, $\omega_{14,15} -43.8$, $\omega_{15,16} 26.5$, $\omega_{16,17} 1.4$, $\omega_{17,13} -28.8^\circ$ in ring D; $\omega_{20,22} -31.2$, $\omega_{22,23} 36.0$, $\omega_{23,24} -27.2$, $\omega_{24,34} 7.6$, $\omega_{34,20} 15.0^\circ$ in the tetrahydrofuran ring. Rings A, B, and C are in chair conformations that depart significantly from ideal to accommodate intramolecular non-bonded 1,3-diaxial interactions involving the Me groups. Ring D torsion angles are related by an approximate mirror plane of symmetry passing through C-14 and the mid-point of the C-16—C-17 bond; thus the ring has an envelope form with C-14 as the out-of-plane atom. The tetrahydrofuran ring has a conformation intermediate between half-chair and envelope forms and, as indicated above, it is involved with the equatorial hydroxy group at C-12 in an intramolecular O . . . HO hydrogen bond [O-34 . . . O-33 = 2.624 (4) Å]. All other hydroxy groups and the H₂O molecules of crystallization participate in a complex hydrogen-bonded network.

Polacandrin [1] demonstrated potent cytotoxicity against KB (ED₅₀ = 0.60 µg/ml), P-388 (ED₅₀ = 0.90 µg/ml), and RPMI-7951 (ED₅₀ = 0.62 µg/ml) tumor cells. It also showed marginal cytotoxic activity against HCT-8 (ED₅₀ = 6.06 µg/ml) colon carcinoma cells.

TABLE 3. Non-Hydrogen Atom Fractional Coordinates for Polacandrin [1] Pentahydrate, with Estimated Standard Deviations in Parentheses.

Atom	x	y	z	Atom	x	y	z
C-1	0.1634(4)	-0.1940(3)	0.4061(1)	C-21	0.1100(5)	0.3919(4)	0.3445(2)
C-2	0.1615(4)	-0.2944(3)	0.4094(1)	C-22	0.2857(4)	0.3230(3)	0.3321(1)
C-3	0.1026(4)	-0.3386(3)	0.3752(1)	C-23	0.3429(4)	0.3776(3)	0.3634(2)
C-4	0.1450(4)	-0.3129(3)	0.3341(1)	C-24	0.3040(3)	0.3427(3)	0.4028(1)
C-5	0.1498(3)	-0.2101(3)	0.3314(1)	C-25	0.2993(4)	0.4059(4)	0.4373(2)
C-6	0.1836(4)	-0.1756(3)	0.2900(1)	C-26	0.4079(5)	0.4404(6)	0.4448(2)
C-7	0.1492(4)	-0.0799(3)	0.2846(1)	C-27	0.2538(5)	0.3630(4)	0.4748(2)
C-8	0.1912(4)	-0.0171(3)	0.3176(1)	C-28	0.0723(5)	-0.3491(4)	0.3014(2)
C-9	0.1741(3)	-0.0595(3)	0.3602(1)	C-29	0.2508(4)	-0.3590(4)	0.3277(2)
C-10	0.2068(3)	-0.1585(3)	0.3657(1)	C-30	0.0144(3)	0.0588(3)	0.3224(1)
C-11	0.2127(4)	0.0050(3)	0.3930(1)	O-31	0.2189(4)	-0.1609(2)	0.4401(1)
C-12	0.1595(3)	0.0942(3)	0.3910(1)	O-32	-0.0029(3)	-0.3134(2)	0.3767(1)
C-13	0.1740(3)	0.1355(3)	0.3496(1)	O-33	0.2043(3)	0.1477(2)	0.4429(1)
C-14	0.1321(3)	0.0728(3)	0.3171(1)	O-34	0.2005(2)	0.3089(2)	0.3942(1)
C-15	0.1444(4)	0.1308(3)	0.2795(1)	O-35	0.2396(4)	0.4831(2)	0.4266(1)
C-16	0.1055(4)	0.2218(3)	0.2933(1)	O-W1	0.4066(3)	0.1265(3)	0.4503(1)
C-17	0.1231(4)	0.2251(3)	0.3399(1)	O-W2	0.4165(4)	0.1831(4)	0.5308(1)
C-18	0.3069(4)	-0.0016(3)	0.3081(1)	O-W3	0.1141(5)	-0.0934(5)	0.5058(2)
C-19	0.3251(4)	-0.1710(4)	0.3663(1)	O-W4	0.0291(4)	0.4767(3)	0.4575(2)
C-20	0.1794(3)	0.3102(3)	0.3513(1)	O-W5	0.441(1)	0.335(2)	0.5524(4)

²Fractional atomic coordinates for polacandrin [1] pentahydrate have been deposited at the Cambridge Crystallographic Data Centre and can be obtained on request from Dr. Olga Kennard, University Chemical Laboratory, 12 Union Road, Cambridge CB2 1EZ, UK.

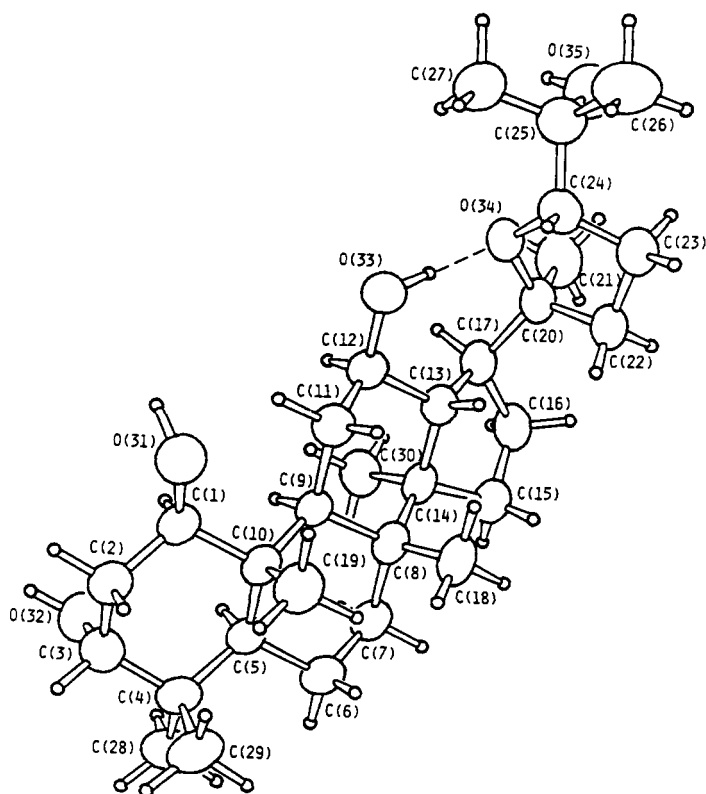


FIGURE 3. ORTEP diagram (50% probability ellipsoids) showing the atom numbering scheme and solid-state conformation of polacandrin [1] in crystals of the pentahydrate. Small circles represent hydrogen atoms.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were taken on a Fischer-Johns melting point apparatus and are uncorrected. Ir spectra were recorded on a Perkin-Elmer 1320 spectrophotometer and refer to KBr pellets. Mass spectra were determined on a VG 70-250 SEQ mass spectrometer. ^1H - and ^{13}C -nmr spectra were measured on Varian XL-400 and Bruker AC 300 spectrometers with TMS as an internal standard. Si gel (Kieselgel 60, 230–400 mesh, Merck) and reversed-phase Si gel (Bondapak C_{18} , 15–20 μ , Waters) were used for cc. Precoated Si gel plate (Kieselgel 60 F₂₅₄ 0.25 mm, Merck) and C_{18} reversed-phase bonded Si gel plates (Baker Si- C_{18}F , 0.2 mm, J. T. Baker) were used for analytical tlc; Si gel GF (1 mm, Analtech) was used for preparative tlc. Detection of polacandrin was made by spraying with 50% H_2SO_4 solution containing 1% anisaldehyde in 95% EtOH, followed by heating.

PLANT MATERIAL.—*P. dodecandra* was collected at Fort Sill Military Reservation, Oklahoma, in August 1990. A voucher specimen is deposited in the Herbarium of the Department of Botany at the University of Oklahoma at Norman.

EXTRACTION AND ISOLATION OF POLACANDRIN [1].—The air-dried herbs (2500 g) were extracted with hot MeOH. The residue after evaporation of the MeOH was digested with H_2O , and the aqueous suspension was extracted with *n*-hexane, CHCl_3 , and *n*-BuOH, successively. The *n*-BuOH-soluble fraction was then chromatographed on Si gel in EtOAc with increasing polarity of MeOH. The active fraction (MeOH) was further purified by flash chromatography on reversed-phase Si gel employing $\text{H}_2\text{O}/\text{MeOH}$ (1→0 and the 0→1 ratio, respectively) as eluent. Further repeated flash chromatography afforded 200 mg (0.008% yield) of polacandrin [1], which was purified by recrystallization from MeOH/ H_2O .

POLACANDRIN [1] PENTAHYDRATE.—Colorless prisms, mp 234–238°, $[\alpha]_{\text{D}} -86.5^\circ$ ($c = 0.89$, CHCl_3); R_f 0.35 (Me_2CO); ir 3400, 3340, 2960, 2870, 1453, 1380, 1367, 1305, 1083, 1030, 1010,

870 cm^{-1} ; ^1H nmr see Table 1; ^{13}C nmr see Table 2; eims m/z $[\text{M} - \text{H}_2\text{O}]^+$ 474.3666 ($\text{C}_{30}\text{H}_{50}\text{O}_4$, calcd 474.3709), 415 (34%), 397 (48), 379 (39), 349 (6), 297 (10), 253 (6.5), 235 (9), 223 (7), 219 (11), 205 (14.5), 189 (16), 159 (19), 143 (100), 125 (68), 107 (34), 95 (29), 85 (32), 71 (36), 59 (25.5); fabms m/z $[\text{M} + 1]^+$ 493.3900 ($\text{C}_{30}\text{H}_{53}\text{O}_5$, calcd 493.3893), 185 (10%), 143 (100), 93 (34.5).

ACETYLATION OF 1.—Compound **1** (40 mg) in dry pyridine (2 ml) and Ac_2O (2 ml) was stored at room temperature overnight. After evaporation of solvent, the residue was chromatographed by preparative tlc employing EtOAc to give diacetate **2** (35 mg): mp 228–230°; R_f 0.34 (EtOAc); ir 3522, 3360, 3280, 2970, 2890, 1725, 1450, 1385, 1375, 1240, 1030, 1005, 962, 872 cm^{-1} ; ^1H nmr see Table 1; ^{13}C nmr see Table 2; fabms m/z $[\text{M} + 1]^+$ 577.4082 ($\text{C}_{34}\text{H}_{57}\text{O}_7$, calcd 577.4104), 559 (9%), 439 (11), 421 (8), 201 (7), 185 (11), 159 (8), 143 (100), 125 (13), 107 (15.5), 93 (26.5).

OXIDATION OF 2.—To a solution of **2** (20 mg) in Me_2CO (10 ml), Jones' reagent was added dropwise with stirring at 0° until a permanent orange color persisted for 2 min. After stirring for an additional 20 min, the reaction mixture was evaporated to remove Me_2CO . The residue was dissolved in ice- H_2O (15 ml), following which the mixture was extracted with Et_2O . The extract was evaporated to dryness, and the residue was recrystallized from $\text{Et}_2\text{O}/n$ -hexane to afford **3** (15 mg) as prisms: mp 254–255°; R_f 0.78 (EtOAc); ir 2990, 2980, 2865, 1770, 1725, 1700, 1455, 1370, 1325, 1240, 1155, 1120, 1020, 990 cm^{-1} ; ^1H nmr see Table 1; ^{13}C nmr see Table 2; fabms m/z $[\text{M} + 1]^+$ 531.3352 ($\text{C}_{31}\text{H}_{47}\text{O}_7$, calcd 531.3322), 471 (39%), 411 (67.5), 395 (34), 277 (10), 257 (7), 229 (8), 207 (16), 185 (37), 161 (27), 149 (30.5), 135 (35), 121 (29), 107 (38), 99 (23), 93 (100).

X-RAY CRYSTAL STRUCTURE ANALYSIS OF POLACANDRIN PENTAHYDRATE [1**] $\cdot 5\text{H}_2\text{O}$.**—Crystal data: $\text{C}_{30}\text{H}_{52}\text{O}_5 \cdot 5\text{H}_2\text{O}$, mol wt = 582.82, orthorhombic, space group $C222_1$ (D_2^5 -No. 20), $a = 13.001$ (2), $b = 15.160$ (2), $c = 33.255$ (4) Å (from 25 orientation reflections, $36^\circ < \theta < 40^\circ$), $V = 6554$ (3) Å³, $Z = 8$, $D_c = 1.181$ $\text{g}\cdot\text{cm}^{-3}$, $\mu(\text{CuK}\alpha$ radiation, $\lambda = 1.5418$ Å) 6.7 cm^{-1} ; crystal dimensions: 0.12 \times 0.30 \times 0.34 mm.

Preliminary unit cell parameters and space group information were derived from oscillation and Weissenberg photographs. One octant of intensity data was recorded on an Enraf-Nonius CAD-4 diffractometer [CuK α radiation, graphite monochromator; ω -2 θ cans; scanwidth (1.00 + 0.14 tan θ)°; $\theta_{\text{max}} = 75^\circ$]. The intensities of four reference reflections, monitored every 2 h during data collection, showed no significant variation (<1%) throughout. The data were corrected for the usual Lorentz and polarization effects; an empirical absorption correction [$T_{\text{max}}:T_{\text{min}} = 1.00:0.90$] was also applied. From a total of 3716 independent measurements, those 2723 reflections with $I > 3.0\sigma(I)$ were retained for the analysis.

The crystal structure was solved by direct methods (MULTAN 11/82). Approximate coordinates for the carbon and oxygen atoms of **1** were derived in part from an E map and from weighted Fourier syntheses phased successively by an increasing number of atoms. Five water oxygen atoms, four of which were clearly defined while the other was quite diffuse, were located in a difference Fourier synthesis evaluated following several rounds of full-matrix least-squares adjustment of the positional and isotropic thermal parameters of the atoms of **1**. In the subsequent least-squares calculations $\{\sum w\Delta^2 [w = 1/\sigma^2(|F_o|), \Delta = (|F_o| - |F_c|)]\}$ was minimized} positional and anisotropic temperature factors of all nonhydrogen atoms were refined. In the later iterations, hydrogen atoms, except those of three H_2O molecules for which their positions were ambiguous, were incorporated at their calculated positions, and an extinction correction (g) was varied. The refinement converged (max. shift: $\text{ESD} = 0.02$) at $R = \sum ||F_o| - |F_c|| / \sum |F_o| = 0.059$, $R_w = [\sum w(|F_o| - |F_c|)^2 / \sum w|F_o|^2]^{1/2} = 0.083$, $g = 4(1) \times 10^{-7}$, $\text{GOF} = [\sum w(|F_o| - |F_c|)^2 / (N_{\text{observations}} - N_{\text{parameters}})]^{1/2} = 2.09$. A final difference Fourier synthesis contained no unusual features (max. $\Delta\rho = 0.30$ $\text{e}/\text{Å}^3$).

Crystallographic calculations were performed on PDP 11/44 and Micro VAX computers by use of the Enraf-Nonius Structure Determination Package (SDP). For structure-factor calculations, neutral atom scattering factors and their anomalous dispersion corrections were taken from the literature (10).

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