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TERPENOID CONSTITUENTS OF *OXANDRA ASBECKII*

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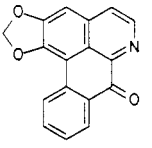
ABSTRACT.—Three C_{31} triterpenoids, formally derived from 24-methyllanostane, have been identified in the leaves of *Oxandra asbeckii*. The structures of two of these compound were assigned as **3**, 2,3-dioxo-oxandrane, and **4**, 3-hydroxyoxandrane, on the basis of the spectroscopic characteristics of the natural products and their derivatives. Two-dimensional nmr methods, especially 2- and 3-bond ^{13}C-^{1}H shift-correlation experiments using the FLOCK pulse sequence, were of paramount importance for these assignments. The third triterpenoid, 3-hydroxyoxandran-2-one [**5**], was obtained, after acetylation of the crude residue, as its acetate **8**. A series of nOe difference measurements carried out on **8** established the main stereochemical features of the oxandrane skeleton. The leaves also afforded the alkaloid liriodenine and the sesquiterpene spathulenol.

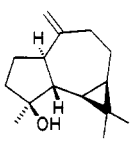
Oxandra asbeckii (Pulle) R.E. Fries (Annonaceae), known locally as "karishiri" or lancewood, is a tree with a restricted range in central Guyana. An extract of the leaves has provided the alkaloid liriodenine [**1**], the sesquiterpene spathulenol [**2**], and three novel methyltriterpenes **3**, **4**, and **5**.

The identity of liriodenine [**1**], which is considered to be an ubiquitous alkaloid of the Annonaceae, was established by comparison of its physical properties with those reported in the literature (1). The structure of spathulenol [**2**] was deduced from its nmr spectra. Its relative configuration was established by the close correspondence of its physical properties, particularly its ^{13}C-nmr spectrum, with those reported previously (2).

The structures of the novel triterpenoids were investigated spectroscopically, and



1



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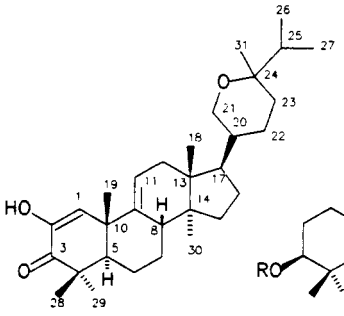
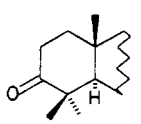


3

4 R=H
6 R=Ac



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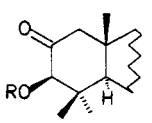

5 R=H
8 R=Ac

the assignments made depended heavily on 2D nmr spectroscopy. Carbon multiplicities were determined from APT spectra, while a standard HETCOR experiment established one-bond ^{13}C-^{1}H connectivities, effectively labeling protonated carbons with their attached hydrogens. Our FLOCK pulse sequence was then used to determine longer-range *n*-bond connectivities (3). The wealth of 2- and 3-bond connectivities observed was more than sufficient to establish the structures of the compounds in hand. Tables 1 and 2 summarize the ^{13}C and ^{1}H chemical shifts that have been assigned. The stereochemistry was determined by nOe difference measurements. The results reported below established that the triterpenoids isolated differ only in ring A. For convenience we have used the name oxandrane to describe the hypothetical parent compound with ring A at the oxidation level of cyclohexane. Few triterpenes have been reported from the Annonaceae, but the most common, polycarpol, has been assigned a structure based on the lanostane skeleton (1). More recently, a $C_{31}H_{50}O$ constituent of *Artabotrys*

TABLE 1. ^{13}C Chemical Shifts of Triterpenoids **3**, **4**, **6**, **7**, and **8**.[a]

Carbon	Compound				
	3	**4**	**6**	**7**	**8**
C-1	124.89	36.08	35.79	36.72	51.67
C-2	143.15	27.77	24.14	34.86	204.47
C-3	200.75	78.90	80.78	217.10	83.84
C-4	43.71	39.10	38.00	47.70	45.24
C-5	50.16	52.46	52.57	53.41	52.61
C-6	27.13	28.13	28.03	27.74	27.63
C-7	21.14	21.33	21.21	22.54	21.50
C-8	41.17	41.69	41.69	41.80	41.84
c-9	143.83	148.57	148.21	147.17	146.56
C-10	40.90	39.40	39.28	39.36	43.28
C-11	115.84	114.69	114.94	116.06	115.64
C-12	36.47	36.62	36.65	36.69	36.51
C-13	44.10	44.09	44.11	44.10	44.19
C-14	47.11	46.94	46.94	46.93	47.00
C-15	33.80	33.76	33.77	33.76	33.80
C-16	26.73	26.76	26.77	26.75	26.89
C-17	48.03	48.07	48.06	48.05	48.01
C-18	14.71	14.69	14.70	14.73	14.80
C-19	24.38	22.19	22.24	21.73	22.90
C-20	39.24	39.26	39.30	39.11	39.31
C-21	65.57	65.63	65.61	65.62	65.57
C-22	26.16	26.15	26.18	26.15	26.26
C-23	33.02	33.02	33.00	33.02	33.11
C-24	74.87	74.82	74.75	74.84	74.83
C-25	39.34	39.36	39.35	39.27	39.43
C-26	16.75	16.75	16.76	16.76	16.89
C-27	17.15	17.15	17.13	17.15	17.29
C-28	22.24	15.66	16.80	22.05	17.65
C-29	25.82	28.24	28.14	25.62	28.99
C-30	18.54	18.52	18.53	18.47	18.69
C-31	14.56	14.57	14.67	14.62	14.73
MeC=O	—	—	170.82	—	170.30
CH_3CO	—	—	21.28	—	20.81

[a]Data obtained at 100.6 MHz with $CDCl_3$ solutions. Assignments based on 1-bond and *n*-bond ^{13}C-^{1}H shift-correlation experiments, except that the *n*-bond correlations were not carried out for **8**. The shift-correlation experiments for **4** were carried out with a pyridine-d_5 solution at 50°; the data reported here for comparison purposes were obtained for a dilute $CDCl_3$ solution.

TABLE 2. ^{1}H Chemical Shifts of Triterpenoids **3**, **4**, **6**, **7**, and **8**.[a]

Proton	Compound				
	3	**4**	**6**	**7**	**8**
H-1	6.58	1.80, 1.43	1.80, 1.52	2.12, 1.83	<2.61>
H-2	—	<1.93>	1.76, 1.66	2.72, 2.40	—
H-3	—	3.21	4.48	—	4.93
H-5	1.68	0.86	0.95	1.73	1.59
H-6	1.70, 1.43	1.64, 1.33	1.64, 1.32	1.69, 1.33	1.70, 1.42
H-7	<1.67>	1.67, 1.46	1.66, 1.47	<1.67>	<1.80>
H-8	2.30	2.15	2.15	2.22	2.16
H-11	5.46	5.19	5.20	5.26	5.15
H-12	2.02, 1.78	2.00, 1.73	2.01, 1.72	2.03, 1.78	2.03, 1.72
H-15	<1.38>	<1.36>	<1.36>	<1.42>	<1.39>
H-16	1.87, 1.32	1.85, 1.32	1.85, 1.32	1.88, 1.34	1.87, 1.32
H-17	1.57	1.56	1.56	1.54	1.59
H-18	0.69	0.66	0.66	0.70	0.67
H-19	1.28	1.03	1.05	1.23	1.04
H-20	1.46	1.46	1.46	1.47	1.48
H-21	3.85, 3.25	3.85, 3.24	3.83, 3.24	3.85, 3.25	3.83, 3.24
H-22	1.67, 1.22	1.68, 1.25	1.68, 1.25	1.72, 1.31	1.66, 1.22
H-23	1.50, 1.40	1.38, 1.24	1.51, 1.35	1.64, 1.40	1.50, 1.36
H-25	1.60	1.61	1.60	1.62	1.61
H-26	0.88	0.89	0.88	0.90	0.89
H-27	0.84	0.85	0.85	0.86	0.85
H-28	1.08	0.81	0.88	1.07	0.86
H-29	1.19	0.98	0.85	1.06	1.10
H-30	0.73	0.72	0.72	0.74	0.76
H-31	1.02	1.03	1.03	1.04	1.03
Ac	—	—	2.04	—	2.18

[a]Data obtained at 400 MHz with $CDCl_3$ solutions. Footnote to Table 1 describes basis for assignments. The δ_H for each identifiable proton (or methyl) is listed. The <mean δ_H> is reported for incompletely resolved CH_2 multiplets.

odorotissimus has been reported to have the lanostane skeleton with the extra carbon appearing as a methylene substituent at C-24 (4).

2,3-Dioxo-oxandrane [**3**] was isolated as white crystals, mp 205–206°, and the formula $C_{31}H_{48}O_3$ was determined by hrms. In the ir the absorption at 1676 cm^{-1} indicated an α,β-unsaturated cyclohexanone, and OH-stretching absorption at 3444 and 3400 cm^{-1} was also observed. Connectivity data (Table 3) established the main skeletal features. The 2- and 3-bond connectivities to the protons of methyl singlets were particularly valuable because they effectively associate identifiable structural units with each methyl group. The overlap between these units was then sufficient for the lanostane framework to be assigned unequivocally. The structure of the side chain, incorporating the extra methyl (C-31) at C-24 and a tetrahydropyran ring, also followed from the observed connectivities. Connectivities to C-24 (δ_C 74.87) are of importance because they establish the location of the terminal isopropyl unit and the C-31 methyl; the observed connectivity through the oxygen to a proton (δ_H 3.85) on C-21 (δ_C 65.57) confirmed the structural assignments to the heterocyclic ring. Connectivity data were also used to establish the substitution pattern on ring A. The 3-bond ^{13}C-^1H correlations between position 1 (δ_C 124.89, δ_H 6.58) and the methyl (δ_C 24.38, δ_H 1.28) at C-10 were observed in both directions; C-1 also showed a 3-bond connectivity to the proton of an OH group on C-2.

TABLE 3. Nmr and Connectivity Data for 2,3-Dioxo-oxandrane [3].

Position	δ_C[a]	δ_H[b]	Observed n-bond connectivity[c]
1	124.89	6.58	6.07, 1.28
2	143.15	—	6.58, 6.07
3	20.75	—	6.58, 1.19, 1.08
4	43.71	—	1.19, 1.08
5	50.16	1.68	6.58, 1.28, 1.19, 1.08
6	27.13	1.70, 1.43	2.30, 1.68
7	21.14	1.67	1.68
8	41.17	2.30	5.46, 0.73
9	143.83	—	1.67, 1.28
10	40.90	—	1.68, 1.28
11	115.84	5.46	2.02, 1.78
12	36.47	2.02, 1.78	5.46, 0.69
13	44.10	—	0.73, 0.69
14	47.11	—	1.78, 0.73, 0.69
15	33.80	1.38	0.73
16	26.73	1.87, 1.32	
17	48.03	1.57	0.69
18	14.71	0.69	
19	24.38	1.28	6.58, 1.68
20	39.24	1.46	3.85, 1.50
21	65.57	3.85, 3.25	1.67, 1.46
22	26.16	1.67, 1.22	3.85
23	33.02	1.50, 1.40	1.02
24	74.87	—	3.85, 1.22, 1.02, 0.84
25	39.34	1.60	1.02, 0.88, 0.84
26	16.75	0.88	0.84
27	17.15	0.84	0.88
28	22.24	1.08	1.19
29	25.82	1.19	1.08
30	18.54	0.73	1.38
31	14.56	1.02	
OH	—	6.07	

[a]^{13}C Chemical shift for $CDCl_3$ solution; 100.6 MHz

[b]^{1}H Chemical shift for $CDCl_3$ solution; 400 MHz. Direct connectivity to C established by HETCOR experiment.

[c]Chemical shift of proton giving cross peak observed at δ_C in FLOCK experiment (3).

The most abundant triterpenoid, $C_{31}H_{52}O_2$, was obtained crystalline, mp 201–202°, and its structure was assigned as 3-hydroxyoxandrane [**4**] by the spectroscopic methods described for **3**. Due to the limited solubility of **4** in $CDCl_3$, it was not possible to obtain a FLOCK spectrum of this compound in $CDCl_3$. Rather, the 2D spectra for structural and initial spectral assignments were measured in pyridine-d_5. Then a one-bond HETCOR spectrum was determined in $CDCl_3$ and ^{1}H and ^{13}C chemical shifts were assigned for **4** in this solvent by comparing with the previously assigned chemical shifts in pyridine-d_5, and with the known chemical shifts for the two derivatives of **4** described below. The stereochemistry at C-3 with the OH equatorial was assigned from the vicinal coupling constants of 10.5 and 5.4 Hz associated with the C-3 proton. Ac_2O in pyridine converted **4** to the acetate **6**, and pyridinium chlorochromate oxidized **4** to oxandran-3-one [**7**]. Both derivatives were crystalline, and each was completely characterized in $CDCl_3$ solution by the spectroscopic methods described above, providing confirmation for the assignments made for **4**. Chemical shift data for **4** in pyridine-d_5, along with the connectivity data used to assign structures and spectra of **4**, **6**, and **7**, are available from the authors at the Toronto address.

The final triterpenoid, 3-hydroxyoxandran-2-one [**5**], was not isolated directly, but it was obtained as the crystalline acetate **8**, $C_{33}H_{52}O_4$, mp 219–220°, by treatment with Ac_2O and pyridine of material from the mother liquors that had afforded **3** and **4**. The amount of compound obtained was not sufficient for the *n*-bond shift-correlation experiment, but one-bond heteronuclear correlations were identified from a HETCOR spectrum. The chemical shift assignments for **8**, Tables 1 and 2, were then made by comparison with the other compounds. The consistency shown for all positions except those in ring A left no doubt that the only difference between **8** and the other triterpenoids isolated was in ring A. It was clear that **8** is an α-acetoxy ketone, and the assignment as the positional isomer shown was based on the observation that the proton, δ 4.93, on the carbon bearing OAc was a singlet, and the ring CH_2 signals showed only geminal coupling; the ^{13}C chemical shifts of the methyls at C-4 were also much more in accord with having an OAc at C-3 (rather than a carbonyl or CH_2). It follows that the parent compound is **5**, but the presence of the acetate in the original extract can not be excluded.

A series of nOe difference measurements was carried out on **8** in order to determine the stereochemistry. Irradiation of either the C-18 or C-19 protons produced a positive nOe (ca. 6%) at H-8, in accord with the lanostane stereochemistry, which has all three moieties on the same (β) face of the molecule. No nOe was observed between the C-18 and C-30 methyl protons, but irradiation of the C-30 methyl singlet produced a positive nOe (ca. 5%) at H-17, again confirming the lanostane stereochemistry with both C-30 and H-17 on the α face. A positive nOe (ca. 4%) was observed for the protons of the methyl assigned as C-28 on irradiation of the C-19 methyl protons, confirming the assignments made for C-28 and C-29. Irradiation of the C-29 methyl protons gave a 7% enhancement of the H-3 signal, requiring H-3 to be α and the OAc to be β. The carbocyclic skeleton of **8** thus has the lanostane stereochemistry, and the side chain probably also has the lanostane configuration, but present data fall short of proving this point. However, the relative configurations around the tetrahydropyran ring can be demonstrated. There is a transdiaxial coupling (11 Hz) between H-20 and the H-21 proton at δ 3.24; this proton (δ 3.24) showed a positive nOe when the C-31 methyl signal was irradiated. Consequently, the C-31 methyl and H-20 have an anti relationship, and both are axial on the heterocyclic ring.

Because of the consistencies exhibited in the nmr of the compounds listed in Tables 1 and 2, the same stereochemistry can reasonably be assigned to all of them. That is to say, the oxandranes are substituted lanostanes.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mp's were determined on a micro hot stage. Selected ir absorptions (Ft-ir) obtained on a Nicolet 5DX spectrometer are reported in cm^{-1}. Nmr spectra were obtained for $CDCl_3$ solutions (unless otherwise indicated); ^1H spectra were obtained at 400 MHz and ^{13}C spectra at 100 MHz on a Varian XL-400 spectrometer. A VG 70-250S mass spectrometer, operating at 70 eV, was used to obtain mass spectra; *m/z* values (with intensity at % of base peak) are reported for significant peaks. Optical rotations were measured on a Perkin-Elmer 243B polarimeter.

PLANT MATERIAL.—Plants were collected in the Essequibo region of Guyana in November 1987. Voucher specimens are deposited in the Herbarium of the University of Guyana and at the Institute of Systematic Botany, University of Utrecht, Netherlands.

EXTRACTION.—Dried, ground leaves (1.1 kg) were extracted with EtOH (15 liters). The solvent was removed under reduced pressure, and the residue was triturated with $CHCl_3$. The $CHCl_3$ solution afforded a residue that was partitioned between hexanes and MeOH-H_2O (9:1). H_2O was added to the MeOH phase until it was 30% aqueous, and then it was extracted with CH_2Cl_2 (3 × 100 ml). The CH_2Cl_2 solution was extracted with diluted HCl, and the acidic extract, after basification, afforded liriodenine [**1**] as yellow needles (6 mg), mp 281–284°.

The hexane phase afforded a residue that was chromatographed on SiO_2 gel with elution by hexanes/Me_2CO mixtures of increasing polarity.

2,3-Dioxo-oxandrane [**3**].—Compound **3** was isolated from early fractions and, after rechromatography (7% Me_2CO/hexanes) and recrystallization from MeOH/CH_2Cl_2, was obtained as white crystals (30 mg): mp 205–206°; $[\alpha]_D$ +75° (c = 0.2, $CHCl_3$); ir (KBr) 3400, 1676; eims 468 (12), 453 (52), 425 (100), 411 (30), 383 (16), 311 (16), 271 (20), 205 (35); hreims 468.3604, calcd for $C_{31}H_{48}O_3$, 468.3603.

3-Hydroxyoxandrane [**4**].—Compound **4** was isolated from later fractions and recrystallized from MeOH/CH_2Cl_2 to provide white crystals (186 mg): mp 201–202°; $[\alpha]_D$ +83° (c = 0.24, pyridine); ir (KBr) 3336, 1038; eims 456 (1), 441 (9), 413 (100), 395 (51), 313 (20), 273 (28), 175 (35), 135 (47); hreims 456.3967, calcd for $C_{31}H_{52}O_2$, 456.3967. Monoacetate **6**: mp 191–192°; $[\alpha]_D$ +80° (c = 0.09, $CHCl_3$); ir (KBr) 1733, 1246, 1039; eims 498 (3), 483 (12), 480 (7), 455 (100), 438 (9), 423 (22), 395 (68), 335 (20); hreims 498.4073, calcd for $C_{33}H_{54}O_3$, 498.4073. Oxidation of **4** (56 mg) with PCC in CH_2Cl_2 at room temperature for 4 h converted it to oxandran-3-one [**7**], which was recrystallized from MeOH/CH_2Cl_2 and obtained as white crystals: mp 212–213°; $[\alpha]_D$ −7.5° (c = 0.3, $CHCl_3$); ir (KBr) 1711; hreims 454.3811, calcd for $C_{31}H_{50}O_2$, 454.3811.

Mother liquors from fractions that provided **3** and **4** were evaporated, treated with Ac_2O/pyridine, and subjected to preparative SiO_2 tlc [hexanes-Me_2CO (2:1)] to afford **8** (8 mg) and **2** (25 mg).

3-Acetoxyoxandran-3-one [**8**]: mp 219–220° (from CH_2Cl_2/MeOH); $[\alpha]_D$ −8° (c = 0.4, $CHCl_3$); ir (KBr) 1751, 1729, 1239; eims 512 (2), 497 (6), 469 (100), 451 (10), 427 (12), 409 (31), 367 (14), 135 (32); hreims 512.3866, calcd for $C_{33}H_{52}O_4$, 512.3866.

Spathulenol [**2**] was obtained as a colorless gum: $[\alpha]_D$ +14.5° (c = 0.3, $CHCl_3$); ir 3443; eims 220 (14), 205 (90), 187 (40), 177 (26), 159 (72), 147 (69), 119 (100), 105 (90); hreims 220.1827, calcd for $C_{15}H_{24}O$, 220.1827.

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