

New Modified Labdane Diterpenoids from *Cluytia richardiana*

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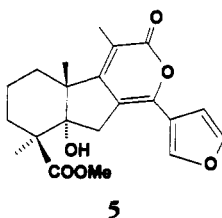
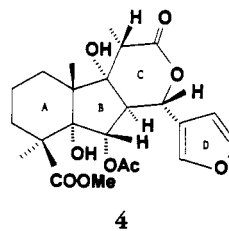
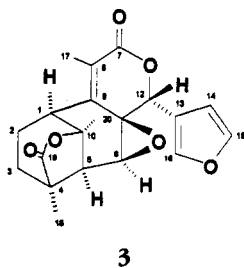
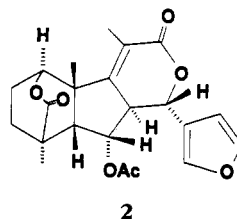
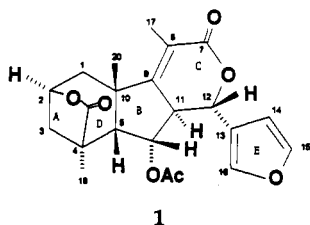
NEW MODIFIED LABDANE DITERPENOIDS
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ABSTRACT.—The aerial parts of *Cluytia richardiana* yielded two new labdane-type diterpenoids, named cluytene A [3] and cluytene B [4]. The structural assignments and relative stereochemistry of both compounds were established from 2D nmr spectral data and by X-ray crystallographic analysis. Furthermore, cluytene B [4] was found to undergo a peculiar elimination reaction when treated with 1,5-diazabicyclo[5.4.0]undec-5-ene to give the highly conjugated product 5.

The shrub *Cluytia richardiana* L. (Euphorbiaceae) (known in Arabic as "Sa'oor") is widely distributed in the mountainous regions of Aseer province of Saudi Arabia (1). Earlier investigations have reported on the isolation of the antidiabetic constituent saudin (2), which was found to possess significant hypoglycemic effect in non-alloxanized fasted mice (3). Subsequent phytochemical work on the leaves of this plant yielded a number of interesting diterpenes having a 6,7-*secolabdane* skeletal backbone (2,4). The



6,7-*seco*-6,11-*cyclolabdan*e-type compounds constitute the major constituents isolated from a collection of plant material native to the "Abha" region with richardianidin-1 [**1**] and richardianidin-2 [**2**] being the principal diterpenes (4). Our examination of several collections of the same source has led to the isolation and characterization of the 6(7),9(10)-*biseco*-6(11),1(9)-*bicyclolabdan*e diterpene cluytene A [**3**] and the 6,7-*seco*-6(11)-*cyclolabdan*e derivative cluytene B [**4**], together with the previously reported saudin (2), and **1** and **2**.

RESULTS AND DISCUSSION

The EtOAc precipitate, obtained from the defatted EtOAc extract of *C. richardiana* (see Experimental), was flash chromatographed (5) over Si gel to give, in crystalline form, the diterpenes saudin (2) and [**1-4**] in 0.015%, 0.004%, 0.02%, 0.01% and 0.015% w/w yields, respectively. Cluytene A [**3**] was found to have the molecular formula $C_{20}H_{20}O_6$, as established by negative-ion cims. It possesses a γ -lactone (ν max 1770 cm^{-1} ; δ_C 177.3), an α,β -unsaturated lactone (ν max 1715 cm^{-1} ; δ_C 163.1) and a furan ring (ν max 3110, 1500 and 870 cm^{-1}). Comparison of the 1H - and ^{13}C -nmr spectral data of **3** with those of richardianidin-1 [**1**] [Tables 1 and 2; and Mossa *et al.* (4)] led to the conclusion that **3** contained the 6,7-*seco*-6,11-*cyclolabdan*e skeletal backbone with the additional presence of a C-6(11) epoxide group (δ_C 56.8, d; 56.4, s) and γ -lactone at C-4(10); instead of an acetate at C-6 and γ -lactone at C-4(2) in **1**. The COSY 2D nmr experiment suggested the presence of the system $>CH-CH_2-CH_2-$ in **3** and was confirmed by a 2D nmr HETCOR experiment and other ^{13}C -nmr spectral data, which showed signals at δ_C 38.2 (d), 26.4 and 25.9 ($2\times t$), 45.8 and 82.2 ($2\times s$) assigned to C-1-C-4 and C-10, respectively. In addition, the COSY 2D nmr spectrum also confirmed

TABLE 1. 1H -Nmr Chemical Shift Values (in ppm) and Coupling Constants (Hz, in parentheses) for Diterpenes **3-5**.^a

Proton	Compound		
	3	4	5
1	2.89 d (6.3)	1.91 br dd (4.0, 14.5)	—
2	1.95 m	1.75 m	—
	1.42 m	1.58 m	
3	1.17 m	2.20 td (4.6, 13.6)	—
	0.91 td (6.2, 13.8)	1.32 m	
5	2.58 br d	—	—
6	3.50 d (2.4)	6.04 d (7.9)	3.74 d (15.8)
			2.75 d (15.8)
8	—	2.88 dq (1.9, 6.4)	—
11	—	2.67 dd (7.9, 11.9)	—
12	5.10 s	5.17 d (11.8)	—
14	6.22 dd (1.0, 1.9)	6.46 br dd (1.0, 1.9)	6.74 dd (1.1, 2.2)
15	7.44 br t (1.6)	7.37 br t (1.5)	7.46 dd (1.8, 2.4)
16	7.41 m	7.50 m	7.83 br s
17	2.08 s	1.25 d (6.6)	2.05 s
18	1.19 s	0.97 s	1.02 s
20	1.44 s	1.02 s	1.34 s
-OAc	—	1.74 s	—
-COOMe	—	3.67 s	3.73 s
OH	—	4.23 br d (2.0)	—
		3.21 br d (2.4)	

^aSpectra for **3** and **4** recorded at 300 MHz and for **5** at 200 MHz.

TABLE 2. ¹³C-Nmr Spectral Data for Diterpenoids **1** and **3-5**.^{ab}

Carbon	Compound			
	1	3	4	5
1	38.6	38.2	31.4	30.3
2	75.0	26.4	18.6	18.9
3	38.7	25.9	27.3	28.9
4	41.8	45.8	48.6 ^c	47.5
5	53.2	48.5	82.1 ^d	83.8
6	71.9	56.8	75.5	36.5
7	165.8	163.1	167.0	164.5
8	120.8	131.7	40.1	116.2 ^e
9	160.9	143.7	84.4 ^d	146.7
10	44.9	82.2	49.9 ^e	53.5
11	51.4	56.4	60.4	115.0 ^e
12	74.2	77.2	73.0	164.5
13	121.3	121.1	121.0	119.8
14	108.7	108.0	109.0	108.2
15	143.6	144.6	143.3	143.7
16	142.1	140.4	142.6	142.0
17	12.9	12.8	9.1	11.3
18	19.6	19.1	18.5	20.3
19	180.0	177.3	175.5	176.3
20	30.2	25.2	23.3	24.0
OAc	20.5	—	20.5	—
-COOMe	168.6	—	173.8	52.0

^aSpectra recorded for **1**, **3** and **4** at 75 MHz, and for **5** at 50 MHz.

^bMultiplicities of the carbon signals of all compounds were determined by APT and DEPT experiments, also aided by 2D nmr COSY and HETCOR experiments.

^cInterchangeable signals.

the presence of the system -CH-CH-O- in **3**. Thus, based on the foregoing data the carbon skeleton of cluytene A was tentatively proposed as 6(7), 9(10)-*biseco*-6(11), 1(9)-*bicyclolabdane*. X-ray crystallographic analysis established the complete structure and relative stereochemistry of **3**. The asymmetric unit consists of three crystallographically independent molecules for which carbon and oxygen atom fractional coordinates¹ are listed in Table 3. Corresponding bond lengths in each of the molecules agree well and all lie close to their expected values (6). The solid-state conformation of one of the molecules is illustrated in Figure 1 [endocyclic torsion angles ω_{ij} (0.3–0.8°) about the bonds between atoms *i* and *j* follow: $\omega_{1,2}$ -41.2, $\omega_{2,3}$ 39.5, $\omega_{3,4}$ -55.4, $\omega_{4,5}$ 69.8, $\omega_{5,10}$ -73.8, $\omega_{1,10}$ 60.9° in the γ -lactone-bridged cyclohexane ring A; $\omega_{1,10}$ -60.9, $\omega_{10,5}$ 49.6, $\omega_{5,6}$ -18.9, $\omega_{6,11}$ -1.0, $\omega_{11,9}$ -11.6, $\omega_{9,1}$ 42.3° in cyclohexane ring B; $\omega_{7,8}$ 16.8, $\omega_{8,9}$ -3.8, $\omega_{9,11}$ -31.1, $\omega_{11,12}$ 51.6, $\omega_{12,25}$ -40.5, $\omega_{25,7}$ 8.0° in α,β -unsaturated δ -lactone ring C; $\omega_{4,5}$ -40.8, $\omega_{5,10}$ 39.9, $\omega_{10,22}$ -23.3, $\omega_{22,19}$ -3.9, $\omega_{19,4}$ 28.8° in γ -lactone ring D; $\omega_{13,14}$ 0.5, $\omega_{14,15}$ 0.0, $\omega_{15,21}$ -0.5, $\omega_{21,16}$ 0.9, $\omega_{16,13}$ -0.9° in furan ring E]. Ring A is in a chair conformation flattened significantly around C-2 and highly puckered around C-5, rings B and C approximate to half-chair and 1,3-diplanar forms, respectively, while ring D has an envelope form with C-5 as the out-of-plane atom. The conformations of the fused rings in the other two molecules are, as would be anticipated, very similar to those in the

¹Atomic coordinates for **3** and **4** have been deposited at the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK.

TABLE 3. Non-hydrogen Atom Fractional Coordinates for the Three Molecules of **3** in the Asymmetric Crystal Unit, with Estimated Standard Deviations in Parentheses.

Atom	x	y	z	Atom	x	y	z
C-1	0.1369(1)	0.3844(4)	-0.3113(3)	C-14	0.0134(2)	0.2345(6)	-0.1117(3)
C-2	0.1646(1)	0.2642(5)	-0.2238(2)	C-15	0.0244(2)	0.1159(7)	-0.0441(3)
C-3	0.1513(1)	0.1039(5)	-0.2632(2)	C-16	-0.0041(2)	0.0308(5)	-0.2110(3)
C-4	0.1527(1)	0.0801(5)	-0.3842(3)	C-17	0.0886(2)	0.6352(5)	-0.1928(4)
C-5	0.1104(1)	0.1898(4)	-0.4601(2)	C-18	0.1455(2)	-0.0838(5)	-0.4187(3)
C-6	0.0489(1)	0.1868(4)	-0.4483(2)	C-19	0.2105(1)	0.1445(5)	-0.3931(2)
C-7	-0.0110(1)	0.5279(4)	-0.2743(3)	C-20	0.1300(2)	0.4607(5)	-0.5099(3)
C-8	0.0525(1)	0.5166(4)	-0.2629(3)	O-21	0.0142(1)	-0.0110(4)	-0.1036(3)
C-9	0.0733(1)	0.4075(4)	-0.3149(2)	O-22	0.2041(1)	0.2945(3)	-0.4130(2)
C-10	0.1427(1)	0.3364(4)	-0.4261(3)	O-23	0.2571(1)	0.0848(4)	-0.3825(2)
C-11	0.0306(1)	0.2961(4)	-0.3767(2)	O-24	0.0135(1)	0.3141(3)	-0.4967(2)
C-12	-0.0204(1)	0.2671(4)	-0.3280(3)	O-25	-0.0463(1)	0.4100(3)	-0.3124(2)
C-13	-0.0051(1)	0.1807(4)	-0.2220(3)	O-26	-0.0340(1)	0.6415(4)	-0.2532(2)
C-1'	0.1765(1)	-0.2688(4)	0.1815(2)	C-14'	0.2868(1)	-0.5810(4)	0.3894(3)
C-2'	0.1847(1)	-0.2582(4)	0.3077(2)	C-15'	0.3134(2)	-0.5744(5)	0.4968(3)
C-3'	0.2421(1)	-0.1815(4)	0.3690(2)	C-16'	0.3725(2)	-0.4748(5)	0.4116(3)
C-4'	0.2553(1)	-0.0426(4)	0.3078(2)	C-17'	0.1348(2)	-0.5903(5)	0.1123(4)
C-5'	0.2573(1)	-0.0845(4)	0.1893(2)	C-18'	0.3084(2)	0.0408(5)	0.3741(3)
C-6'	0.2985(1)	-0.2101(4)	0.1855(2)	C-19'	0.1994(1)	0.0491(4)	0.2798(3)
C-7'	0.2384(2)	-0.6445(4)	0.1079(2)	C-20'	0.1719(2)	-0.1052(4)	0.0126(3)
C-8'	0.1975(1)	-0.5340(4)	0.1286(2)	O-21'	0.3667(1)	-0.5083(4)	0.5145(2)
C-9'	0.2137(1)	-0.3935(3)	0.1560(2)	O-22'	0.1632(1)	0.0000(-) ^a	0.1839(2)
C-10'	0.1927(1)	-0.1191(4)	0.1371(2)	O-23'	0.1857(1)	0.1495(3)	0.3322(2)
C-11'	0.2769(1)	-0.3627(4)	0.1693(2)	O-24'	0.2898(3)	-0.2809(3)	0.0785(2)
C-12'	0.3172(1)	-0.4901(4)	0.2128(2)	O-25'	0.2962(1)	-0.6223(3)	0.1482(2)
C-13'	0.3258(1)	-0.5166(4)	0.3330(2)	O-26'	0.2229(1)	-0.7572(3)	0.0550(2)
C-1''	0.3654(1)	-0.0694(4)	-0.2217(2)	C-14''	0.5241(2)	-0.4323(5)	-0.2470(3)
C-2''	0.3471(1)	-0.1991(5)	-0.3047(2)	C-15''	0.5200(2)	-0.4800(6)	-0.3486(3)
C-3''	0.3601(1)	-0.3532(5)	-0.2532(3)	C-16''	0.5144(2)	-0.2363(6)	-0.3553(3)
C-4''	0.3468(1)	-0.3657(4)	-0.1403(2)	C-17''	0.4200(2)	0.1778(5)	-0.3325(4)
C-5''	0.3819(1)	-0.2471(4)	-0.0625(2)	C-18''	0.3523(2)	-0.5254(5)	-0.0962(3)
C-6''	0.4465(1)	-0.2461(4)	-0.0554(2)	C-19''	0.2851(1)	-0.3048(5)	-0.1575(3)
C-7''	0.5183(1)	0.0804(4)	-0.2230(3)	C-20''	0.3523(1)	0.0276(5)	-0.0383(3)
C-8''	0.4538(1)	0.0668(4)	-0.2501(3)	O-21''	0.5135(2)	-0.3622(5)	-0.4188(2)
C-9''	0.4305(1)	-0.0398(4)	-0.2004(2)	O-22''	0.2879(1)	-0.1542(3)	-0.1434(2)
C-10''	0.3493(1)	-0.1069(4)	-0.1137(2)	O-23''	0.2393(1)	-0.3678(4)	-0.1833(2)
C-11''	0.4701(1)	-0.1431(4)	-0.1237(2)	O-24''	0.4774(1)	-0.1128(3)	-0.0062(2)
C-12''	0.5269(1)	-0.1731(4)	-0.1524(3)	O-25''	0.5524(1)	-0.0296(3)	-0.1697(2)
C-13''	0.5223(1)	-0.2723(5)	-0.2494(3)	O-26''	0.5429(1)	0.1906(4)	-0.2465(2)

^aThe y-coordinate of O(22') was held constant throughout the analysis to define the space group origin in this direction.

molecule shown but the orientations of their essentially planar furan rings differ significantly in response to crystal packing effects [torsion angles defining the orientation about the C-12–C-13 bond are C-11–C-12–C-13–C-16=87.6(4)° and O-25–C-12–C-13–C-14=32.4(4)° for the molecule shown in Figure 1; corresponding pairs of angles in the other molecules are -101.8(4)°, -49.9(4)° and 92.2(4)°, 151.5(3)°].

Cluytene B [**4**], C₂₃H₃₀O₉, possessed ¹H- and ¹³C-nmr spectral data (Tables 1 and 2) that were generally similar to those of richardianidin-1 [**1**] (**4**), except for the presence of two tertiary hydroxyl groups (δ_C 82.1, 84.4), a secondary methyl group (δ_H 1.25, 3H, d, J=6.6 Hz) and a methyl ester (δ 3.67, 3H, s, δ_C 52.0), instead of the vinylic methyl group at C-17 and C-4(2)-γ-lactone in **1**. A 2D nmr COSY experiment showed that the signal at δ 2.67 (H-11) was coupled to two different oxymethine protons at δ 6.04 and 5.17 (H-6 and H-12, respectively), thus confirming the presence of the system -O-CH-CH-CH-O- in **4**. Because all the ¹H- and ¹³C-nmr assignments for richardianidins **1** and **2** (**1**) and (**2**) have been unambiguously established (4), the placement of the two tertiary hydroxyl groups of **4** at C-5 and C-9 was straight-forward. An X-ray crystallographic analysis defined the entire structure and relative stereochemistry of **4**. Non-hydrogen atom fractional coordinates¹ are provided in Table 4. Bond lengths and angles are in accord with expectations (6). A view of the solid-state conformation is presented

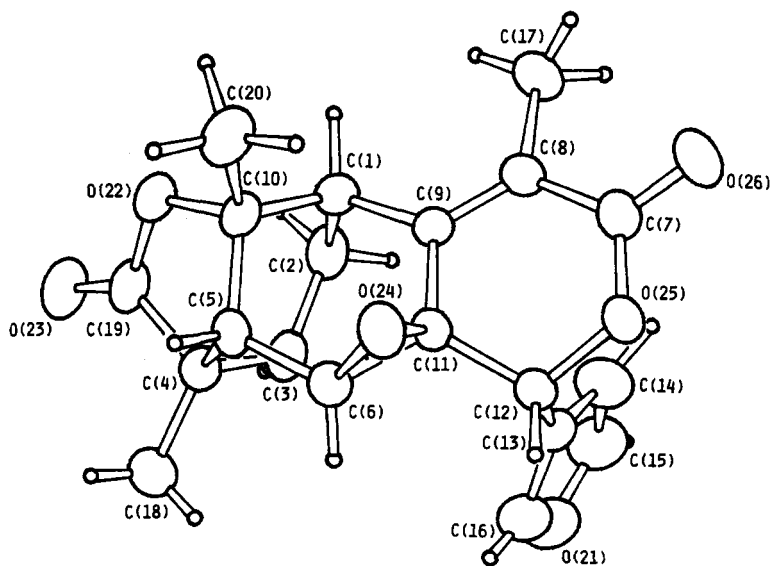


FIGURE 1. ORTEP Diagram (40% Probability Ellipsoids) Showing the Crystallographic Atom Numbering Scheme and Solid-state Conformation of One of the Three Molecules of **3** in the Asymmetric Crystal Unit; Small Circles Represent Hydrogen Atoms.

in Figure 2 [endocyclic torsion angles ω_{ij} (0.2 – 0.4°) about the bonds between atoms i and j follow: $\omega_{1,2}$ -52.8 , $\omega_{2,3}$ 53.5 , $\omega_{3,4}$ -53.6 , $\omega_{4,5}$ 57.3 , $\omega_{5,10}$ -59.8 , $\omega_{10,1}$ 55.5° in the cyclohexane ring A; $\omega_{5,6}$ -44.8 , $\omega_{6,11}$ 25.4 , $\omega_{11,9}$ 3.4 , $\omega_{9,10}$ -30.7 , $\omega_{10,5}$ 46.7° in the cyclopentane ring B; $\omega_{7,8}$ -54.7 , $\omega_{8,9}$ 50.8 , $\omega_{9,11}$ 0.3 , $\omega_{11,12}$ -52.3 , $\omega_{12,24}$ 54.2 , $\omega_{24,7}$ 0.6° in the δ -lactone ring C; $\omega_{13,14}$ -1.8 , $\omega_{14,15}$ 3.0 , $\omega_{15,21}$ -3.0 , $\omega_{21,16}$ 1.9 , $\omega_{13,16}$ -0.1° in furan ring D]. Cyclohexane ring A is in a slightly flattened chair conformation, cyclopentane ring B has an envelope form with C-5 as the out-of-plane atom, δ -lactone ring C is in a boat conformation, and the furan ring is essentially planar. Both hydroxy groups participate in hydrogen-bonded interactions. The hydroxy group at C-5 is intramolecularly hydrogen-bonded to that at C-9 [O-22–H-22...O-26, O...O = $2.791(3)\text{\AA}$], while the hydrogen atom of the latter is involved in an intermolecu-

TABLE 4. Non-hydrogen Atom Fractional Coordinates for [4], with Estimated Standard Deviations in Parentheses.

Atom	x	y	z	Atom	x	y	z
C-1	0.2608(2)	0.3690(1)	0.3061(4)	C-17	0.2816(2)	0.4347(1)	-0.1103(4)
C-2	0.2795(3)	0.3209(1)	0.4188(4)	C-18	-0.0475(3)	0.2985(1)	0.5774(4)
C-3	0.1727(3)	0.3084(1)	0.5395(4)	C-19	0.0530(3)	0.2570(1)	0.3255(4)
C-4	0.0538(2)	0.3054(1)	0.4381(4)	C-20	0.1545(2)	0.3258(1)	0.0490(4)
C-5	0.0393(2)	0.3563(1)	0.3306(3)	O-21	-0.4105(2)	0.4157(1)	-0.1373(4)
C-6	-0.0695(2)	0.3664(1)	0.2069(3)	O-22	0.0369(2)	0.3939(1)	0.4723(2)
C-7	0.0818(2)	0.4726(1)	-0.1630(4)	O-23	-0.1769(2)	0.3756(1)	0.3068(3)
C-8	0.1490(2)	0.4274(1)	-0.0887(3)	O-24	-0.0377(2)	0.4691(1)	-0.1574(3)
C-9	0.1036(2)	0.4181(1)	0.1074(3)	O-25	0.1257(2)	0.5099(1)	-0.2269(3)
C-10	0.1435(2)	0.3664(1)	0.1977(3)	O-26	0.1329(2)	0.4608(1)	0.2186(3)
C-11	-0.0341(2)	0.4157(1)	0.1089(3)	O-27	0.1217(2)	0.2225(1)	0.3442(3)
C-12	-0.0874(2)	0.4229(1)	-0.0797(4)	O-28	-0.0370(2)	0.2553(1)	0.2076(3)
C-13	-0.2187(2)	0.4299(1)	-0.0827(4)	C-29	-0.0388(3)	0.2113(1)	0.0902(5)
C-14	-0.2863(3)	0.4694(1)	-0.0030(5)	C-30	-0.2754(2)	0.3492(1)	0.2603(5)
C-15	-0.3993(3)	0.4604(1)	-0.0431(6)	O-31	-0.2745(2)	0.3134(1)	0.1591(5)
C-16	-0.2966(3)	0.3988(1)	-0.1614(5)	C-32	-0.3826(3)	0.3690(2)	0.3488(6)

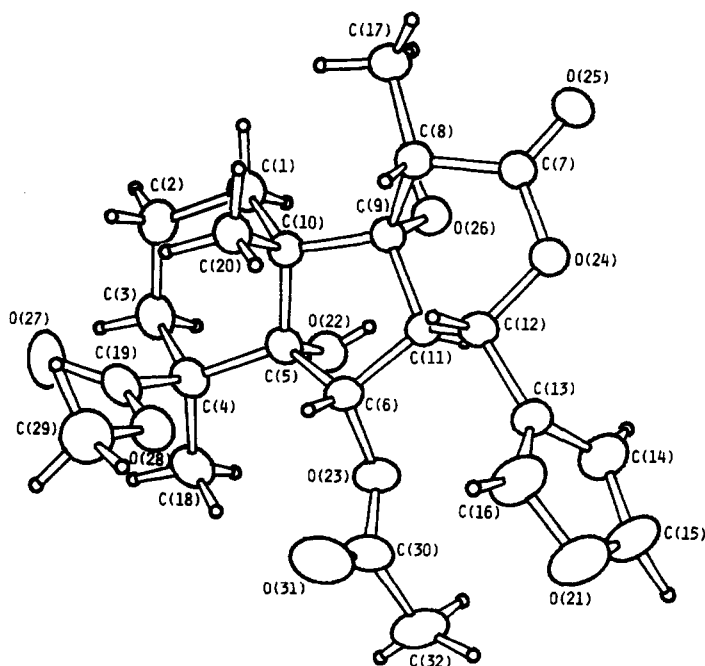


FIGURE 2. ORTEP Diagram (40% Probability Ellipsoids) Showing the Crystallographic Atom Numbering Scheme and Solid-state Conformation of **4**; Small Circles Represent Hydrogen Atoms.

ar hydrogen-bonded interaction between molecules related by the crystallographic 2_1 screw axis along the *c*-direction [$O-26 \dots O-25 = 2.859(3)\text{\AA}$].

Treatment of **4** with 1,5-diazabicyclo[5.4.0]undec-5-ene led to the formation of the anhydro-derivative **5**, which is probably derived from an intermediate epoxide as shown in Figure 3. The ^{13}C -nmr spectrum of **5** (Table 2) exhibited signals for four quarternary carbons at δ_{C} 116.2, 146.7, 115.0 and 164.5 (C-8, C-9, C-11 and C-12) due to the presence of double bonds at the C-8(9) and C-11(12) positions, respectively. The ^{13}C -nmr data, including the HETCOR spectra, also confirmed the anticipated formation of a vinylic methyl group at C-17 (δ 2.05, 3H, s; δ_{C} 11.3), together with the absence of the C-6-OAc group found in **4**.

Cluytene A [**3**] and cluytene B [**4**] possess an obvious structural relationship to other 6,7-*seco*-6(11)-cyclolabdane diterpenes previously isolated from *C. richardiana* (**4**). However, the biogenetic pathway for **3** involves extensive oxidation, scission of the C-6(7) and

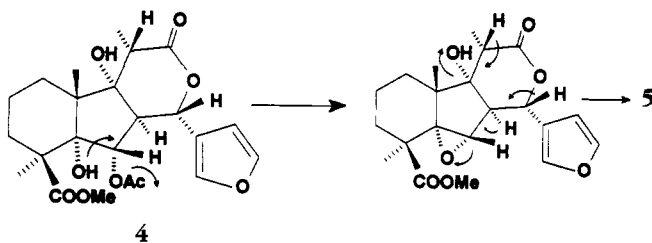


FIGURE 3. Dehydration of **4** to **5** by 1,5-Diazabicyclo[5.4.0]undec-5-ene.

C-9(10) bonds (2) and the rearrangement of the B-ring of the furanoid labdane precursor to give the unique C-6(11), 1(9)-*bicyclo*-skeleton of cluytene A **[3]**. Further work is in progress on the biological evaluation of these constituents.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mp: uncorrected. Uv: EtOH, ir: KBr. The nmr spectra were taken on Varian instruments at 300 (or 200) MHz (^1H) and 75 (or 50) MHz (^{13}C) in CDCl_3 using tetramethylsilane (TMS) as internal standard. Multiplicity determinations (APT and DEPT/or DEPTGL) and 2D nmr spectra (COSY and HETCOR) were obtained using standard Varian software. Negative-ion and positive-ion cims were recorded on a Finnigan 3300 mass spectrometer, using NH_3 as ionizing gas. Optical rotations were recorded in CHCl_3 at ambient temperature using a Perkin-Elmer 241 MC polarimeter. Tlc was performed on Si gel 60 F 254, using CHCl_3 - Me_2CO (9:1) as solvent, with visualization by 1% vanillin/ H_2SO_4 spray reagent.

PLANT MATERIAL.—The aerial parts (leaves and stems) of *Cluytia richardiana* L. (Euphorbiaceae) were collected in Abha, Saudi Arabia in June 1991. A voucher specimen is deposited at the herbarium of MAPPRC, College of Pharmacy, King Saud Univeristy, Riyadh, Saudi Arabia.

EXTRACTION AND ISOLATION OF DITERPENOID.—The dried ground aerial parts of the plant material (3.2 kg) were percolated successively at room temperature with petroleum ether (bp 60–80°) then EtOAc (yields 78 g and 129 g, respectively). On concentration *in vacuo*, the crude EtOAc extract afforded a greenish-white precipitate, which was filtered off (yields 55 g and 74 g, respectively, for the precipitate and mother liquor solubles). A portion of the EtOAc precipitate (48 g) was subjected to cc over Si gel (1.4 kg), eluting initially with petroleum ether (bp 60–80°), then with increasing concentrations of EtOAc in petroleum ether. Elution with petroleum ether-EtOAc (8.5:1.5) afforded cluytene A **[3]**, 346 mg, R_f 0.64, solvent: CHCl_3 - Me_2CO (9:1), followed by saudin (473 mg, R_f 0.60, mp 245–246°, $[\alpha]_D - 113^\circ$ ($c=0.01$, CHCl_3), ^1H - and ^{13}C -nmr data were indistinguishable from those reported in the lit. (2)²), richardianidin-2 (**[2]**, 676 mg, R_f 0.54, mp 262–264° and $[\alpha]_D - 1^\circ$ ($c=0.01$, CHCl_3); lit. (4) mp 266–268° and $[\alpha]_D - 0.02^\circ$) and richardianidin-1 (**[1]**, 125 mg, R_f 0.50, mp 222–224° and $[\alpha]_D + 10^\circ$ ($c=0.01$, CHCl_3); lit. (4) mp 220–222° and $[\alpha]_D + 8^\circ$). Compounds **1** and **2** were identified from their spectral data and by direct comparison with their respective authentic samples. Further elution with petroleum ether-EtOAc (4:1) yielded cluytene B (**[4]**, 436 mg, R_f 0.30) as colorless plates.

Cluytene A [3].—Colorless needles from petroleum ether/EtOAc; mp 262–263°; $[\alpha]_D - 150^\circ$ ($c=0.08$, CHCl_3); uv λ max 210 (log ϵ 4.05) and 270 (3.60) nm; ir ν max 3110, 1770 (γ -lactone), 1715 (α,β -unsaturated lactone), 1500, 1455, 1370, 1250, 1160, 1130, 1070, 925, 870, and 760 cm^{-1} ; ^1H and ^{13}C nmr, see Tables 1 and 2, respectively; nicims m/z $[\text{M}-\text{H}]^-$ 355 ($[\text{C}_{20}\text{H}_{20}\text{O}_6-\text{H}]^-$, 100), 338 (48) and 311 (m/z 355– CO_2 , 30).

Cluytene B [4].—Colorless plates from hot EtOAc; mp 235–236°; $[\alpha]_D + 15^\circ$ ($c=0.08$, CHCl_3); uv λ max, 210 (log ϵ 4.15) and 255 (3.26) nm; ir ν max 3400 (br, OH), 3110, 1765, 1735 and 1700 ($-\text{CO}-\text{OMe}$, OAc and lactone), 1500, 1460, 1380, 1275, 1220, 1190, 1130, 1090, 1065, 1010, 955, 870, and 750 cm^{-1} ; ^1H and ^{13}C nmr, see Tables 1 and 2, respectively; cims m/z $[\text{MH}]^+$ 451 ($[\text{C}_{23}\text{H}_{30}\text{O}_9+\text{H}]^+$, <1), 408 ($[\text{MH}]^+$ –43, 25), 391 ($[\text{MH}]^+$ –60, 100), 373 (m/z 391– H_2O , 20), 347 (22), 193 (15), 165 (15) and 78 (20).

Dehydration of Cluytene B [4] to 5.—Compound **4** (100 mg) in dry C_6H_6 (10 ml) was refluxed with 1,5-diazabicyclo[5.4.0]undec-5-ene (50 mg) at 90° for 8 h. The reaction mixture was acidified with 0.1 N H_2SO_4 , then diluted with ice and extracted into CHCl_3 , from which the major product **[5]** was obtained by crystallization (petroleum ether/ CHCl_3) as colorless needles (65 mg); mp 214–216°, $[\alpha]_D - 6^\circ$ ($c=0.07$, CHCl_3), uv λ max 210 (log ϵ 4.26) and 320 (3.76) nm; ir ν max 3450 (br OH), 3120, 1720 ($-\text{CO}-\text{OMe}$), 1690 (lactone), 1650 ($\text{C}=\text{C}$), 1590, 1450, 1370, 1220, 1070, 1000, 870, and 810 cm^{-1} ; ^1H and ^{13}C nmr, see Tables 1 and 2, respectively; nicims m/z $[\text{M}-\text{H}]^-$ 371 ($[\text{C}_{21}\text{H}_{24}\text{O}_6-\text{H}]^-$, 100) and 353 ($[\text{M}-\text{H}]^- - \text{H}_2\text{O}$, 20).

X-RAY CRYSTAL STRUCTURE ANALYSES OF CLUYTENE A [3] AND CLUYTENE B [4].—Crystal data for **[3]**: $\text{C}_{20}\text{H}_{20}\text{O}_6$; MW=356.38, monoclinic, space group $\text{P}2_1(\text{C}_2^2)$ -No. 4 from the systematic absences $0k0$ when $k \neq 2n$ and **3** is chiral, $a=23.666(2)$, $b=8.983(1)$, $c=12.539(1)$ Å, $\beta=105.17(1)^\circ$, $V=2572.8$ Å³, $Z=6$, $D_c=1.380$ g cm^{-3} , $\mu(\text{CuK}\alpha$ radiation, $\lambda=1.5418$ Å)=8.1 cm^{-1} ; crystal dimensions: $0.06 \times 0.12 \times 0.60$ mm.

Crystal data for **[4]**: $\text{C}_{23}\text{H}_{30}\text{O}_9$; MW=450.49, orthorhombic, space group $\text{P}2_12_12_1(\text{D}_2^4)$ —No. 19 uniquely from the systematic absences $b00$ when $b \neq 2n$, $0k0$ when $k \neq 2n$, $00l$ when $l \neq 2n$, $a=11.279(1)$,

²The mp and $[\alpha]_D$ of saudin were incorrectly reported by Mossa *et al.* (2).

$b=26.447(2)$, $c=7.358(1)\text{\AA}$, $V=2194.9(7)\text{\AA}^3$, $Z=4$, $D_c=1.363\text{ g cm}^{-3}$, $\mu(\text{CuK}\alpha\text{ radiation})=8.4\text{ cm}^{-1}$; crystal dimensions: $0.27\times 0.30\times 0.40\text{ mm}$.

Oscillation and Weissenberg photographs yielded preliminary unit-cell parameters and space group information. Intensity data (5632 non-equivalent $+h, -k, \pm l$ reflections for **3**; 2618 non-equivalent $+h, +k, +l$ reflections for **4**) were recorded on an Enraf-Nonius CAD-4 diffractometer [CuK α radiation, graphite monochromator; ω - 2θ scans, θ max = 75° ; scanwidth $(1.00+0.14\tan\theta)^\circ$ for **3**, $(0.60+0.14\tan\theta)^\circ$ for **4**]. The intensities of four reference reflections from each crystal, re-measured after every 2 h during data collection, showed insignificant variation ($<1\%$). The usual Lorentz and polarization corrections were applied to the intensity data, and those 4296 and 2215 reflections for **3** and **4**, respectively, with $I>3.0\sigma(I)$ were retained for the structure analyses.

Both crystal structures were solved by direct methods (MULTAN11/82). Approximate coordinates for all non-hydrogen atoms of **4** were obtained from an E -map. For compound **3**, the asymmetric unit consists of three crystallographically independent molecules. Coordinates for 24 out of a total of 72 non-hydrogen atoms were derived from an initial E -map, and the remaining atoms were located in a series of weighted F_o Fourier syntheses phased successively by an increasing number of atoms. After several rounds of full-matrix least-squares adjustment of positional and thermal parameters (at first isotropic and then anisotropic) of the carbon and oxygen atoms, difference Fourier syntheses yielded hydrogen atom positions. In the subsequent least-squares iterations, hydrogen atom positional and isotropic thermal parameters were included as variables. For **4**, an extinction correction g was also refined during the final least-squares cycles. The parameter refinements converged (max. shift: $\text{esd}=0.02$) at $R=0.40$ ($R_w=0.053$, $\text{GOF}=1.09$) for **3** and $R=0.039$ [$R_w=0.056$, $\text{GOF}=1.63$, $g=1.8(2)\times 10^{-6}$] for **4**, where $R=\frac{\sum||F_o|-|F_c||}{\sum|F_o|}$; $R_w=\frac{[\sum w(|F_o|-|F_c|)^2]}{\sum w|F_o|^2}^{1/2}$; $\text{GOF}=\frac{[\sum w(|F_o|-|F_c|)^2]}{(N_{\text{observations}}-N_{\text{parameters}})}^{1/2}$.

Crystallographic calculations were performed on PDP11/44 and MicroVAX computers by use of the Enraf-Nonius Structure Determination Package (SDP). For all structure-factor calculations, neutral atom scattering factors and their anomalous dispersion corrections were taken from the literature (7). In the least-squares iterations, $\sum w\Delta^2$ [$w=1/\sigma^2(|F_o|)$, $\Delta=(|F_o|-|F_c|)$] was minimized.

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LITERATURE CITED

1. A.M. Migahid, "Flora of Saudi Arabia, 1," King Saud University Library, Riyadh, Saudi Arabia, 1989, 3rd ed., p. 135.
2. J.S. Mossa, J.M. Cassady, M.D. Antoun, S.R. Byrn, A.T. McKenzie, J.F. Kozolowski, and P. Main, *J. Org. Chem.*, **50**, 916 (1985).
3. J.S. Mossa, E.S.M. El-Denshary, R. Hindawi, and A.M. Ageel, *Int. J. Crude Drug Res.*, **26**, 81 (1988).
4. J.S. Mossa, J.M. Cassady, J.F. Kozolowski, T.M. Zennie, M.D. Antoun, M.G. Pellechia, A.T. McKenzie, and S.R. Byrn, *Tetrahedron Lett.*, **29**, 3627 (1988).
5. W.C. Still, M. Khan, and A. Mitra, *J. Org. Chem.*, **43**, 2923 (1978).
6. F.H. Allen, O. Kennard, D.G. Watson, L. Brammer, A.G. Orpen, and R. Taylor, *J. Chem. Soc., Perkin Trans. II*, S1 (1987).
7. J.A. Ibers and W.C. Hamilton, eds., "International Tables for X-ray Crystallography," The Kynoch Press, Birmingham, UK, Vol. IV, 1974.

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