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NEW WITHANOLIDES FROM *JABOROSA MAGELLANICA*

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ABSTRACT.—Chilean *Jaborosa magellanica* has supplied a series of four new withanolides that incorporate a γ-lactone side chain as well as an extra hemiketalic ring. These withanolides are (−)-jaborotetrol [**1**], (−)-jaborolone [**5**], and the chlorinated (+)-jaborochlorotriol [**6**] and (−)-jaborochlorodiol [**8**]. A newly found pair of withanolides possessing a δ-lactone side chain are (+)-jaboromagellone [**11**] and (+)-projaborol [**9**]. The latter could function as a possible precursor for the previously known (+)-jaborol [**10**].

Approximately 130 withanolides are presently known. These are steroidal compounds each incorporating 28 carbon atoms. They are found among the botanical genera *Withania, Acnistus, Dunalia, Physalis, Nicandra, Datura, Trechonaetes*, and *Jaborosa*, all of which belong to the Solanaceae. Some of the withanolides have demonstrated antibacterial, antitumor, and anti-inflammatory activity (1).

Systematic chemical studies of four *Jaborosa* species, namely *Jaborosa integrifolia* (2–4), *Jaborosa leucotricha* (5), *Jaborosa bergii* (6), and *Jaborosa magellanica* (7,8), have previously been reported. These resulted in the isolation and characterization of many γ-lactone species as well as jaborosalactones A, B, C, D, E, F, and L, all of which incorporate a δ-lactone system bonded to the steroidal skeleton. We have now found six new withanolides in *J. magellanica* (Griseb.) Dusen, four of which incorporate a γ-lactone side chain (structures **1**, **5**, **6–8**) and two of which possess a δ-lactone side chain (structures **9** and **11**) (7). In order to avoid confusion in naming *Jaborosa* withanolides, we have avoided the alphabetically based nomenclature followed in the past. This nomenclature has resulted in two structurally different (+)-jaborosalactone M's being reported in the literature in 1988 (6,8). In order to clear up this potentially confusing situation, it is suggested that the two (+)-jaborosalactone M's be henceforth referred to as (+)-jaborosalactone M-magellanica and (+)-jaborosalactone M-bergii, thus indicating their different character.

Our first withanolide, (−)-jaborotetrol [**1**], $C_{28}H_{38}O_8$, showed only terminal uv absorption at 220 nm. The ir spectrum indicated an unsaturated five-membered lactone (1735 cm^{-1}), as well as an unsaturated ketone (1675 cm^{-1}).

The ^1H nmr spectrum in $CDCl_3$ has been summarized around expression **1**. Of very special interest, beyond the presence of the γ-lactone system, is the six-membered cyclic hemiketal arrangement between what must have originally been a C-12 ketonic function and a C-22 alcohol. The six-membered hemiketal ring was also found in all of our other *Jaborosa* withanolides whenever a γ-lactone side chain was present (9).

Directly related to the presence of the unsaturated γ-lactone and the six-membered hemiketal in (−)-jaborotetrol [**1**] are the nmr signals at δ 4.04 (dd) and 4.83 (br s) attributable to the axial H-22 and the lactone ring H-23, respectively (see numbering system in Figure 1). H-22 is in a 1,3-diaxial relationship with the alcoholic functions at C-12 and C-17. This feature is responsible for the pronounced nmr downfield shift of H-22, from δ 4.04 to 4.69, when pyridine-d_5 is substituted for $CDCl_3$ as solvent (see Experimental) (10).

The steric relationship between H-20, H-22, and H-23 in (−)-jaborotetrol [**1**] is further indicated by the patterns of nmr spin-spin couplings. On the one hand $J_{20,22}$ is

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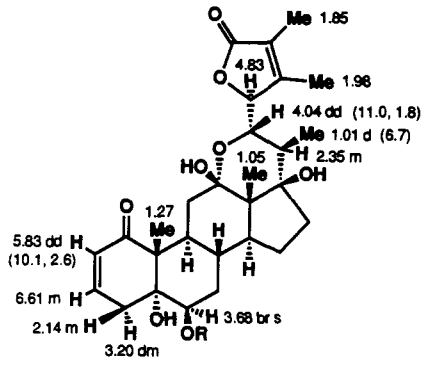
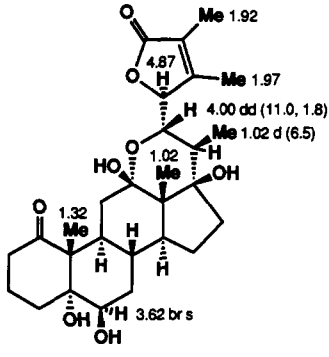


1 R=H (nmr)
2 R=Ac



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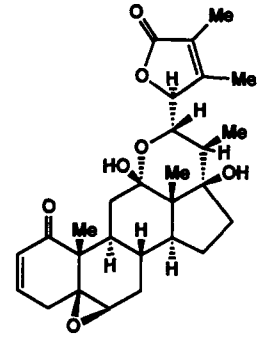

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large, 11.0 Hz, pointing to a trans relationship between H-20 and H-22, with a dihedral angle of approximately 175°. On the other hand, $J_{22,23}$ is small, with a value of 1.8 Hz, denoting a dihedral angle of approximately 60° between H-22 and H-23. Molecular models indicate that this is the sterically least encumbered arrangement for the γ-lactone ring in relation to the rest of the molecule.

A ^{13}C-nmr study in $CDCl_3$ of (−)-jaborotetrol [**1**] (see Experimental), supplemented by a GASPE analysis (11), confirmed the structural assignment. In particular, the carbonyl carbons C-1 and C-26 appear at δ 203.99 and 175.91, respectively, while the hemiketalic C-12 is at δ 102.13.

It is known that the shape of the cd curve between 330 and 340 nm is a reliable indi-

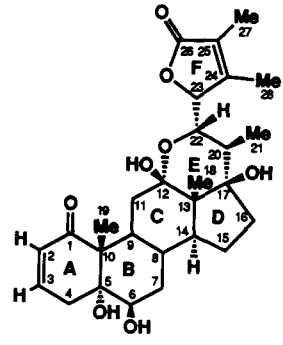
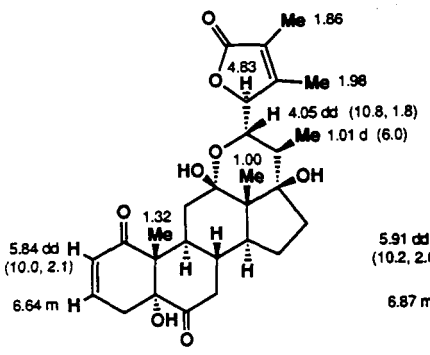


FIGURE 1. Numbering system for withanolides.



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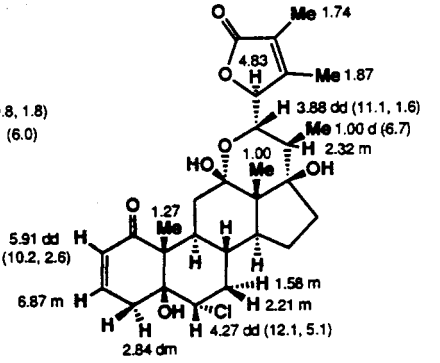

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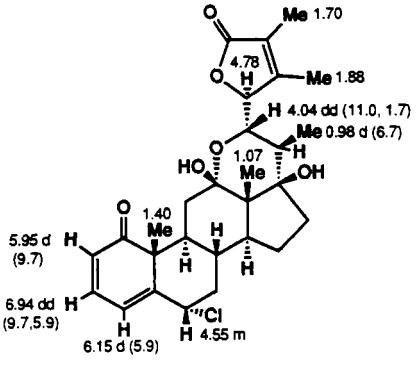

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cator of A/B ring junction in 2-en-1-one steroids. (−)-Jaborotetrol [**1**] displayed a minimum around 330 nm, pointing to a trans-fused arrangement at C-5 and C-10, as indicated (12–14).

Mass measurements were in accord with the structural assignment. In the low resolution mass spectrum, the small molecular ion peak, *m/z* 502 (6%), was accompanied by base peak 152, $C_9H_{12}O_2$. This base peak results from the cleavage of the C-17 (20) and the C-22 to oxygen bonds and represents the γ-lactone moiety together with C-21, C-22, and C-23 and accompanying hydrogens. A strong *m/z* 111 peak for $C_6H_7O_2$ represents the γ-lactone ring and is also present in all of the other withanolides under consideration. These mass spectral assignments were further confirmed by high resolution mass spectral measurements (Experimental section).

Acetylation of (−)-jaborotetrol [**1**] with Ac_2O in pyridine provided (−)-6-*O*-acetyljaborotetrol [**2**], $C_{30}H_{40}O_9$, in which the H-6 nmr signal had shifted significantly downfield from δ 3.68 to 4.79. The trans disposition of the A/B ring fusion in the acetate derivative **2** was again indicated by a cd trough at 330 nm (12–14).

Palladium on carbon reduction of withanolide **1** supplied (+)-2,3-dihydrojaborotetrol [**3**], $C_{28}H_{40}O_8$, whose ^{1}H-nmr spectrum was devoid of vinylic signals.

(−)-Jaborotetrol [**1**] may very well arise biogenetically from the known (−)-jaborosalactone M [**4**] (8) through opening of the oxirane ring with formation of a trans glycol, and it is the main withanolide of *J. magellanica*.

Our second withanolide is (−)-jaborolone [**5**], $C_{28}H_{36}O_8$. The ir spectrum of this material displayed three carbonyl bands at 1740, 1720, and 1680 cm^{-1}. The ^{13}C nmr in $CDCl_3$ and two drops of MeOH, supplemented by a GASPE analysis, showed three

carbonyl peaks at δ 209.10 (C-6), 201.50 (C-1), and 175.46 (C-26). The principal ^{1}H-nmr chemical shifts are summarized around structure **5**. (−)-Jaborolone was fully characterized when it was found to be identical in all respects with the semisynthetic product obtained from the MnO_2 oxidation of (−)-jaborotetrol [**1**].

There are a few examples of chlorinated withanolides within the Solanaceae. Two had been obtained from *J. integrifolia* (3,4), two from *Physalis peruviana* (15,16), one from *Withania frutescens* (17), and one from *Withania somnifera* (18).

J. magellanica, just like *J. integrifolia*, can produce halogenated withanolides specifically incorporating a chlorine atom at C-6. Our third and fourth withanolides are in fact the C-6 chlorinated (+)-jaborochlorotriol [**6**] and (−)-jaborochlorodiol [**8**].

(+)-Jaborochlorotriol [**6**], $C_{28}H_{37}O_7Cl$, displayed a peak in the cd spectrum at 325 nm diagnostic of a cis A/B ring fusion (12–14). Detailed spin decoupling experiments showed that H-6_{ax} (δ 4.27) is split by H-7_{ax} (δ 1.58) with a coupling of 12.1 Hz, as well as by H-7_{eq} (δ 2.21) with a smaller 5.1 Hz coupling. Pyridine nmr shifts were again useful, because the Me-19 singlet underwent a substantial shift from δ 1.27 in $CDCl_3$ to 1.52 in pyridine-d_5 due to the proximity of the C-5 hydroxyl function.

Another indication of the cis fusion for the A/B system was forthcoming from the ^{13}C-nmr spectrum (Experimental). The angular C-19 is found relatively upfield at δ 8.29 due to gauche interaction with the 5β-hydroxyl function (19).

Recrystallization of **6** from MeOH produced the corresponding ketal **7** with a methoxyl rather than a hydroxyl at C-12 ($C_{29}H_{39}O_7Cl$), and an X-ray analysis was carried out on this derivative (Figure 2). The X-ray analysis was carried out by Dr. M. Parvez of the Department of Chemistry, The Pennsylvania State University. The crystals are orthorhombic, *P2,2,2*, $a = 8.944$ (4), $b = 11.906$ (4), $c = 25.443$ (6) Å, $V = 2709$ (3) Å, $Z = 4$, $D = 1.312$ kg/m^3, λ (MoKα) = 0.71073 Å, $\mu = 0.182$ mm^{-1}, $R = 0.040$ for 2337 observed data with $I > 3\sigma(I)$. The results clarified in particular the

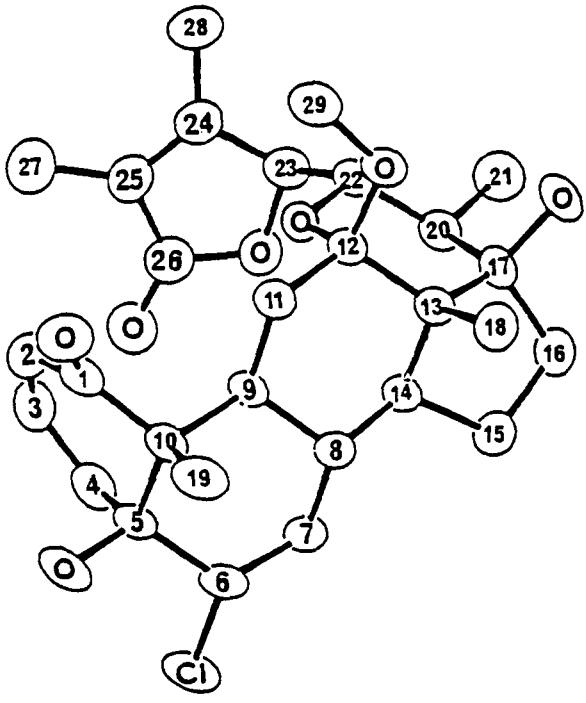


FIGURE 2. Perspective drawing of Compound **7**.

C-23 stereochemistry in relation to C-22. This work will be described in full in a separate paper.

The next withanolide we found was (−)-jaborochlorodiol [**8**] $C_{28}H_{35}O_6Cl$. The ^{1}H-nmr spectrum in $CDCl_3$ exhibited a doublet of doublets at δ 6.94 for H-3, which is coupled both to H-2 (δ 5.95) and H-4 (δ 6.15). The angular C-19 (δ 1.40) is further downfield than in withanolides **1** and **3–6**, and the same holds true for the allylic H-6 (δ 4.55). The uv spectrum showed a maximum at 315 nm as expected for a cis dienone (20). (−)-Jaborochlorodiol [**8**] is probably formed in nature by dehydration of (+)-jaborochlorotriol [**6**].

A note of caution should be inserted here relating to the nature of the two chlorinated withanolides (+)-jaborochlorotriol [**6**] and (−)-jaborochlorodiol [**8**]. There is a possibility but not a certainty that both of these compounds, as well as the previously known chlorinated analogues (15–18), may be artifacts of isolation, formed during cc using the $CHCl_3$/MeOH solvent system. This system contains trace amounts of HCl, which could eventually result in net chlorine uptake by some of the more reactive withanolides.

Turning now to our last two novel δ-lactone withanolides from *J. magellanica*, we first describe (+)-projaborol [**9**], $C_{28}H_{38}O_6$, which may possibly function as the immediate biogenetic precursor of the known (+)-jaborol [**10**], and secondly (+)-jaboromagellone [**11**], which may in turn act as the precursor of (+)-projaborol (7).

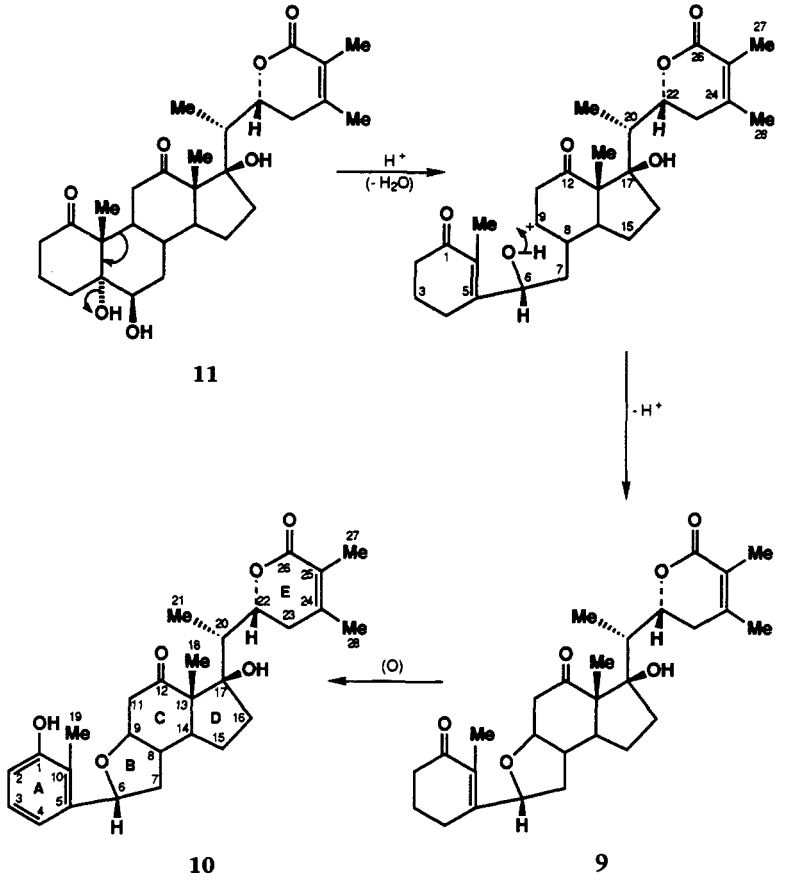
Three intense absorptions were present in the $CHCl_3$ ir spectrum of (+)-projaborol [**9**] at 1650, 1710, and 1740 cm^{-1}, due to the C-1, C-26, and C-12 carbonyl groups, respectively. The 500 MHz ^{1}H-nmr spectrum obtained in $CDCl_3$ solution is indicated around structure **9**. With the notable exception of the chemical shifts for H-6 and those hydrogens attached directly to ring A, the overall spectral pattern closely resembled that for the related (+)-jaborol [**10**] (7). It was clear that a conjugated ketone system was present as a part of ring A in (+)-projaborol [**9**], because the C-2, C-3, and C-4 protons were evident as methylene multiplets at δ 2.10, 1.70, and 1.79 with a total area corresponding to six hydrogens, while the methyl substituent at C-19 appeared as a singlet at δ 2.35, i.e., further downfield than the corresponding signal in (+)-jaborol [**10**], which is present at δ 2.17. Furthermore, the H-6 resonance in (+)-projaborol [**9**] was at δ 5.33, as compared to that in (+)-jaborol [**10**], which is further upfield at δ 4.97 (7).

The mass spectrum of (+)-projaborol [**9**] showed a small molecular ion *m/z* 470. By comparison, (+)-jaborol [**10**] exhibited a small molecular ion *m/z* 468. The difference of two Daltons in the mol wt is consistent with the description of (+)-projaborol [**9**] as an enone, while (+)-jaborol [**10**] is the analogous phenol. The base peak for (+)-projaborol [**9**], *m/z* 109, corresponded to C_7H_9O and is due to ring A. Another significant ion, *m/z* 125 ($C_7H_9O_2$), represented the δ-lactone ring E, due to scission of the C-20 (22) bond.

Finally, the cd curve of (+)-projaborol [**9**] included a maximum at 255 nm, diagnostic of the C-22 *R* configuration as in (+)-jaborol [**10**] (12–14, 21).

Following our isolation and characterization of (+)-projaborol [**9**], we continued with our study of the *J. magellanica* extracts in the hope that we could find out which classical type of withanolide was most likely to function as an in vivo precursor to (+)-projaborol [**9**]. Our efforts led to the isolation and characterization of the new classical type withanolide (+)-jaboromagellone [**11**], $C_{28}H_{40}O_7$. The KBr ir spectrum of this compound showed a broad band between 1620 and 1760 cm^{-1} due to the three carbonyl groups present.

It was possible to assign specific nmr chemical shifts to each of the protons of **11**, because we were able to supplement the information in our 500 MHz ^{1}H spectrum with



SCHEME 1
Possible Biogenetic Pathway

spin decoupling studies, as well as with a ^{13}C-nmr spectrum, a GASPE spectrum, a $^{1}H/^{13}C$ correlation spectrum, a NOESY experiment, and a COLOC analysis (22).

The beta stereochemistry of the hydroxyl groups at C-6 and C-17 was indicated by the appreciable downfield shifts of 0.17 and 0.26 ppm suffered by the C-18 and C-19 methyl singlets, respectively, when pyridine-d_5 was used as solvent in lieu of $CDCl_3$ (10).

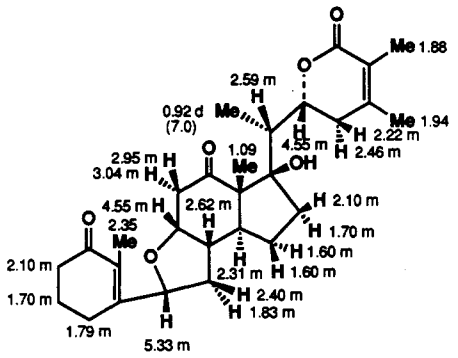
The mass spectrum of (+)-jaboromagellone [**11**] displayed a small molecular ion *m/z* 488, and the familiar base peak *m/z* 125 ($C_7H_9O_2$) representing ring E after the scission of the C-20 (22) bond. Alternate cleavage of the C-17 (20) linkage generated the *m/z* 152 ($C_9H_{12}O_2$) peak.

(+)-Jaboromagellone [**11**] showed a cd spectrum with a maximum at 240 nm indicative of the C-22 *R* configuration (12–14, 21).

Acetylation of **11** using Ac_2O in pyridine led to (+)-6-*O*-acetyljaboromagellone [**12**], $C_{30}H_{42}O_8$. Significantly, the ^{1}H-nmr spectrum of this derivative displayed the H-6 signal at δ 4.84, i.e., appreciably further downfield than in the parent compound **11**, where it appeared at δ 3.72.

Alternately, oxidation of (+)-jaboromagellone [**11**] with MnO_2 in EtOH at room temperature for 24 h furnished (+)-6-oxojaboromagellone [**13**], $C_{28}H_{38}O_7$, whose mass spectrum indicated a molecular ion *m/z* 486.

A possible sequence for the in vivo formation of (+)-jaborol [**10**] is now indicated in Scheme 1. Enzyme-catalyzed cleavage of (+)-jaboromagellone [**11**] can lead to an in-



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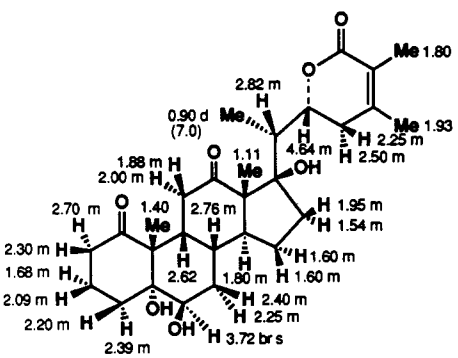

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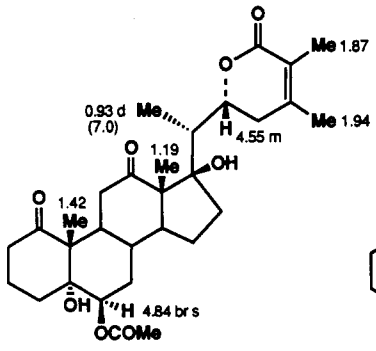

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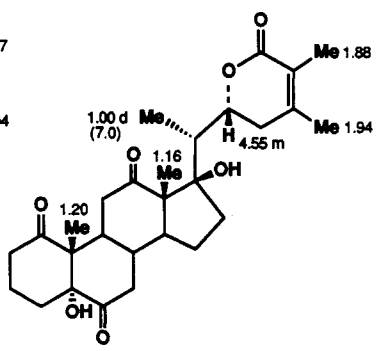

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termediate which can readily cyclize to (+)-projaborol [**9**]. Oxidation and aromatization of ring A then give rise to (+)-jaborol [**10**]. Such a sequence will require proof from in vivo feeding experiments using labeled precursors.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Optical rotations are at 25°. ^{1}H-nmr spectra were recorded on Bruker WM-360 (^{13}C at 90 MHz) and AM-500 (^{13}C at 125 MHz) spectrometers in $CDCl_3$. ^{13}C/^{1}H correlation data and COLOC experiments were collected in the inverse mode. NOe's were calculated as percent maximum possible enhancement, so that a maximum 0.5 enhancement corresponded to 100% nOe. Mass spectral data was collected on a Kratos MS 9/50 using electron impact ionization. Cc was on Merck Kieselgel 60. Tlc was on Merck Si gel glass plates, 0.25 mm thick.

PLANT COLLECTION.—Plant collection was carried out in the summer of 1987, along the shore of Magellan Strait, near Punta Arenas, the most southerly town in Chile. A voucher specimen is deposited in the herbarium of the Universidad de Magallanes.

EXTRACTION AND FRACTIONATION.—The whole dried plant of *J. magellanica* (13.9 kg) was milled and extracted at room temperature with EtOH. The extract was concentrated under reduced pressure to afford an oily mass (1.5 kg). This material was diluted with H_2O and extracted with $CHCl_3$. Evaporation of the organic solvent provided a residue (30 g) of crude withanolides which was then placed on a chromatographic column of Si gel. Elution was with $CHCl_3$ containing increasing amounts of MeOH/EtOAc. Several fractions were obtained which easily crystallized, in spite of their being mixtures of two or three different compounds. Further purification was by tlc on Si gel, especially using the system $CHCl_3$-Me_2CO-MeOH (8:2:1). In the data below, values with identical superscripts are interchangeable.

(−)-JABOROTETROL [**1**].—Compound 1 (500 mg): mp 246–250° (EtOAc); ir ν max (KBr) 3450, 1735, 1675 cm^{-1}; uv λ max (MeOH) 220 nm (log ϵ 3.14); $[\alpha]_D$ −20° (c = 1.07, MeOH); cd MeOH $\Delta\epsilon$ (nm) −0.17 (330); ^{1}H nmr (C_5D_5N, 360 MHz) δ 6.58 (1H, m, H-3), 6.00 (1H, dd, J = 10.1 and 2.2

Hz, H-2), 4.98 (1H, br s, H-23), 4.69 (1H, dd, J = 11.2 and 2.0 Hz, H-22), 4.11 (1H, br s, H-6), 1.81 (3H, s, Me-28), 1.68 (3-H, s, Me-27), 1.58 (3H, s, Me-19), 1.39 (3H, s, Me-18), 1.23 (3H, d, J = 6.6 Hz, Me-21); ^{13}C nmr ($CDCl_3$ + 5 drops of MeOD) δ 203.99 (C-1), 175.91 (C-26), 156.63 (C-24), 142.02 (C-3), 128.08 (C-2), 124.93 (C-25), 102.13 (C-12), 82.73 (C-23), 77.79* (C-5), 77.33* (C-17), 73.72 (C-6), 68.89 (C-22), 51.41 (C-10), 47.63 (C-13), 45.61 (C-14), 38.18 (C-9), 35.59 (C-11), 35.13 (C-20), 35.03 (C-16), 32.39 (C-4), 29.81 (C-7), 29.44 (C-8), 22.66 (C-15), 15.62 (C-19), 12.13 (C-21 and C-28), 10.51 (C-18), 8.32 (C-27); eims *m/z* $[M]^+$ 502 (6), 484 (30), 466 (32), 334 (14), 333 (56), 152 (100), 124 (48), 111 (54). Hreims for $C_{28}H_{36}O_7$ $[M-H_2O]^+$ 484.2453, calcd 484.2461; for $C_{28}H_{32}O_5$, 448.2275, calcd 448.2250; for $C_{19}H_{25}O_5$, 333.1704, calcd 333.1702; for $C_{19}H_{25}O_4$, 317.1783, calcd 317.1753; for $C_{19}H_{21}O_3$, 297.1499, calcd 297.1491; for $C_9H_{12}O_2$, 152.0857, calcd 152.0837; for $C_9H_{11}O_2$, 151.0780, calcd 151.0759; for $C_7H_8O_2$, 124.0537; calcd 124.0524; for $C_6H_7O_2$, 111.0464, calcd 111.0446.

(−)-6-*O*-ACETYLJABOROTETROL [**2**].—Acetylation of (−)-jaborotetrol [**1**] (10.0 mg) with pyridine (0.2 ml) in Ac_2O (0.2 ml) produced (−)-6-*O*-acetyljaborotetrol [**2**]. This compound was purified by tlc using $CHCl_3$-MeOH (95:5): mp 212° (MeOH); ir ν max (KBr) 1730, 1680 cm^{-1}; uv λ max (MeOH) 227 nm (log ε 3.90); [α]D −7° (c = 0.72, MeOH); cd MeOH Δε (nm) −1.23 (330); ^{1}H nmr ($CDCl_3$, 360 MHz) δ 6.53 (1H, m, H-3), 5.83 (1H, dd, J = 10.0 and 2.3 Hz, H-2), 4.86 (1H, br s, H-23), 4.79 (1H, s, H-6), 2.11 (3H, s, Ac), 1.98 (3H, s, Me-28), 1.85 (3H, s, Me-27), 1.22 (3H, s, Me-19), 1.04 (3H, s, Me-18), 1.02 (3H, d, J = 6.6 Hz, Me-21); eims *m/z* (%) $[M]^+$ 544 (5), 508 (100), 420 (6), 415 (16), 375 (30), 209 (16), 125 (30), 124 (11), 111 (28).

PREPARATION OF (+)-2,3-DIHYDROJABOROTETROL [**3**].—(−)-Jaborotetrol [**1**] (10 mg) was reduced with H_2 and 5% Pd/C. The product was purified by tlc, using the system MeCN-C_6H_6-EtOAc-MeOH (40:20:30:2): mp 216° (MeOH); ir ν max ($CHCl_3$) 1740, 1700 cm^{-1}; uv λ max (MeOH) 226 nm (log ε 4.37); [α]D +50° (c = 0.28, MeOH); ^{1}H nmr (C_5D_5N, 360 MHz) δ 4.99 (1H, br s, H-23), 4.63 (1H, dd, J = 11.0 and 2.0 Hz, H-22), 4.05 (1H, s, H-6), 1.86 (3H, s, Me-28), 1.67 (3H, s, Me-27), 1.36 (3H, s, Me-18), 1.21 (3H, d, J = 6.6 Hz, Me-21); eims *m/z* (%) $[M]^+$ 504 (7), 486 (4), 468 (43), 152 (100), 151 (57), 127 (54), 111 (64).

(−)-JABOROLONE [**5**].—Purified by tlc using the system $CHCl_3$-Me_2CO-MeOH (8:2:1) yield (30 mg): mp 264° (MeOH); ir ν max ($CHCl_3$) 1740, 1720, 1680 cm^{-1}; uv λ max (MeOH) 218 nm (log ε 4.03); cd MeOH Δε (nm) −0.16 (350); [α]D −33° (c = 3.55, MeOH); ^{1}H nmr (C_5D_5N, 360 MHz) δ 6.57 (1H, m, H-3), 5.95 (1H, dd, J = 10.2 and 2.3 Hz, H-2), 5.00 (1H, br s, H-23), 4.69 (1H, dd, J = 10.9 and 2.0 Hz, H-22), 1.85 (3H, s, Me-28), 1.66 (3H, s, Me-27), 1.26 (3H, d, J = 6.6 Hz, Me-21); ^{13}C nmr ($CDCl_3$ + 2 drops of MeOD) δ 209.10 (C-6), 201.50 (C-1), 175.46 (C-26), 156.05 (C-24), 141.58 (C-3), 127.72 (C-2), 125.18 (C-25), 101.67 (C-12), 82.52 (C-23), 82.12 (C-5), 79.48 (C-17), 69.05 (C-22), 55.45 (C-10), 47.77 (C-13), 46.31 (C-14), 39.92 (C-11), 37.94* (C-9), 37.43* (C-8), 35.18 (C-20), 33.58 (C-16), 31.21 (C-4), 30.22 (C-7), 22.52 (C-15), 13.80 (C-19), 12.17 (C-21 and C-28), 10.55 (C-18), 8.44 (C-27); eims *m/z* $[M]^+$ 500 (22), 482 (16), 472 (64), 464 (26), 371 (44), 331 (56), 291 (8), 275 (14), 153 (42), 152 (88), 125 (100), 124 (64), 111 (66).

A solution of (−)-jaborotetrol [**1**] (30 mg) in 15 ml of Me_2CO was stirred with MnO_2 (500 mg) at room temperature overnight. The solvent was removed and the product (25 mg) was purified by tlc. The main compound proved to be (−)-jaborolone [**5**], identical with the natural product.

(+)-JABOROCHLOROTRIOL [**6**].—This withanolide (20 mg) acquired a reddish coloration upon spraying with 40% H_2SO_4 and heating on a hot plate: mp 232° (MeOH); ir ν max (KBr) 3450, 1740, 1675 cm^{-1}; uv λ max (MeOH) 233 nm (log ε 3.82); cd MeOH Δε (nm) 0 (360), +0.04 (325), 0 (270), +0.86 (235); [α]D +13° (c = 0.24, MeOH); ^{1}H nmr (C_5D_5N, 360 MHz) δ 7.05 (1H, m, H-3), 6.26 (1H, dd, J = 10.3 and 2.2 Hz, H-2), 4.98 (1H br s, H-23), 4.62 (1H, m, H-6), 4.42 (1H, dd, J = 11.0 and 1.6 Hz, H-22), 2.55 (1H, m, H-20), 1.80 (3H, s, Me-28), 1.63 (3H, s, Me-27), 1.52 (3H, s, Me-19), 1.30 (3H, s, Me-18), 1.23 (3H, d, J = 6.6 Hz, Me-21); ^{13}C nmr ($CDCl_3$ + 3 drops of MeOD) δ 201.87 (C-1), 174.99 (C-26), 156.76 (C-24), 145.39 (C-3), 125.52 (C-2), 123.89 (C-25), 100.79 (C-12), 82.14 (C-23), 79.20 (C-17), 77.08 (C-5), 68.76 (C-22), 65.09 (C-6), 55.14 (C-10), 47.50 (C-13), 45.88 (C-14), 42.05 (C-9), 37.54 (C-11), 34.73* (C-20), 34.31* (C-8), 33.45 (C-16), 31.19 (C-4), 30.18 (C-7), 22.08 (C-15), 11.70 (C-21 and C-28), 10.10 (C-18), 8.29 (C-19), 8.04 (C-27); eims *m/z* $[M]^+$ 520 (9), 502 (11), 416 (71), 398 (53), 350 (16), 316 (65), 152 (100), 125 (98), 111 (47). Recrystallization from MeOH produced ketal **7** which was used in the X-ray crystallography.

(−)-JABOROCHLORODIOL [**8**].—Compound **8** (20 mg): mp 188–190° (MeOH); ir ν max ($CHCl_3$) 3680, 1790, 1660 cm^{-1}; uv λ max (MeOH) 315 nm (log ε 3.96); [α]D −10° (c = 2.04, MeOH); ^{1}H nmr (C_5D_5N, 360 MHz) δ 6.94 (1H, dd, J = 9.7 and 5.7 Hz, H-3), 6.15 (1H, d, J = 5.7 Hz, H-4), 5.95 (1H, d, J = 10.3 Hz, H-2), 4.78 (1H, br s, H-23), 4.55 (1H, br s, H-6), 4.04 (1H, dd, J = 11.0 and 1.7 Hz,

H-22), 1.88 (3H, s, Me-28), 1.70 (3H, s, Me-27), 1.40 (3H, s, Me-19), 1.07 (3H, s, Me-18), 0.98 (3H, d, $J = 6.7$ Hz, Me-21); eims *m/z* $[M]^+$ 504 (3), 502 (3), 335 (24), 333 (48), 277 (21), 264 (90), 153 (100), 151 (63), 123 (75), 111 (69).

(+)-JABOROMAGELLONE [**11**].—Compound **11** (80 mg): mp 289° (MeOH); ir ν max (KBr) 3125, 1760–1620 cm^{-1}; uv λ max (MeOH) 226 nm (log ϵ 3.93); $[\alpha]_D$ +109° ($c = 0.85$, MeOH); cd MeOH $\Delta\epsilon$ (nm) 0 (330), +0.46 (280), +0.35 (262 sh), +0.68 (240); ^1H nmr (C_5D_5N, 360 MHz) δ 5.33 (1H, m, H-22), 4.17 (1H, br s, H-6), 3.54 (1H, m, H-20), 2.40 (1H, m, H-23α), 2.23 (1H, m, H-23β), 1.80 (3H, s, Me-28), 1.66 (3H, s, Me-19), 1.60 (3H, s, Me-27), 1.28 (3H, s, Me-18), 0.90 (3H, d, $J = 7.1$ Hz, Me-21); ^{13}C nmr ($CDCl_3$ + 3 drops of MeOD) δ 214.27 (C-12), 213.61 (C-1), 167.29 (C-26), 150.78 (C-24), 120.95 (C-25), 82.95 (C-17), 78.46 (C-5), 77.80 (C-22), 74.35 (C-6), 59.77 (C-13), 54.00 (C-10), 48.04 (C-14), 42.71 (C-20), 39.46 (C-9), 39.37 (C-7), 36.59 (C-16), 33.18 (C-23), 32.62 (C-11), 32.52 (C-2), 29.81 (C-4), 28.65 (C-8), 22.52 (C-15), 20.26 (C-3), 19.98 (C-28), 17.67 (C-18), 15.80 (C-19), 13.07 (C-21), and 11.69 (C-27); eims *m/z* $[M]^+$ 488 (2), 470 (32), 452 (32), 335 (25), 279 (12), 152 (58), 125 (100). Hreims for $C_{28}H_{40}O_7$, 488.2813, calcd 488.2773; for $C_{28}H_{38}O_6$, 470.2681, calcd 470.2668; for $C_{19}H_{27}O_5$, 335.1860, calcd 335.1858; for $C_{16}H_{23}O_4$, 279.1590, calcd 279.1596; for $C_9H_{12}O_2$, 152.0842, calcd 152.0837; for $C_7H_9O_2$, 125.0608, calcd 125.0602; for C_8H_9O, 121.0656, calcd 121.0653; for C_8H_{13}, 109.1017, calcd 109.1018.

(+)-PROJABOROL [**9**].—Compound **9** (5.6 mg): mp 163° (MeOH); ir ν max ($CHCl_3$) 1740, 1710, 1650 cm^{-1}; uv λ max (MeOH) 285, 220 nm (log ϵ 3.05, 3.09); $[\alpha]_D$ +32° ($c = 1.24$, MeOH); cd MeOH $\Delta\epsilon$ (nm) −1.5 (300), 0 (280), +5.7 (255); ^1H nmr (CD_3CN, 500 MHz) δ 6.82 (1H, br s, OH), 5.30 (1H, m, H-6), 4.65 (1H, m, H-22), 4.52 (1H, m, H-9), 2.98 (1H, m, H-11α), 2.75 (1H, m, H-11β), 2.59 (2H, m, H-7β and H-8), 2.52 (1H, m, H-20), 2.48 (1H, m, H-23α), 2.35 (3H, s, Me-19), 2.28 (1H, m, H-14), 2.20 (1H, m, H-23β), 2.10 (2H, m, H-2), 2.05 (1H, m, H-16β), 1.87 (3H, s, Me-28), 1.79 (2H, m, H-4), 1.78 (3H, s, Me-27), 1.70 (2H, m, H-3), 1.65 (1H, m, H-7α), 1.60 (2H, m, H-15β and H-16α), 1.53 (1H, m, H-15α), 1.00 (3H, s, Me-18), 0.86 (3H, d, $J = 7.0$ Hz, Me-21); eims *m/z* $[M]^+$ 470 (1), 450 (3), 424 (7), 319 (22), 297 (4), 269 (4), 135 (42), 125 (42), 109 (100), 97 (13), 79 (10).

(+)-6-*O*-ACETYLJABOROMAGELLONE [**12**].—(+)-Jaboromagellone [**11**] (5 mg) was treated overnight at room temperature with Ac_2O in pyridine. Workup and tlc gave 3 mg of (+)-6-*O*-acetyljaboromagellone [**12**]: mp 180° (MeOH); ir ν max (KBr) 1740, 1720, 1710, 1690 cm^{-1}; uv λ max (MeOH) 226 nm (log ϵ 3.86); $[\alpha]_D$ +44° ($c = 1.08$, MeOH); cd MeOH $\Delta\epsilon$ (nm) 0 (350), +0.22 (290), +0.18 (272 sh), +0.40 (245); eims *m/z* $[M]^+$ 530 (1), 377 (21), 334 (18), 321 (21), 243 (19), 210 (16), 153 (19), 152 (32), 127 (11), 125 (100).

(+)-6-OXOJABOROMAGELLONE [**13**].—(+)-Jaboromagellone [**11**] (5 mg) was stirred with excess freshly prepared MnO_2 in EtOH at room temperature for 24 h. Workup provided 2 mg of **13**: mp 273° (MeOH); ir ν max ($CHCl_3$) 3480, 1735, 1705, 1690, 1666 cm^{-1}; uv λ max (MeOH) 225 nm (log ϵ 3.57); $[\alpha]_D$ +147° ($c = 0.62$, MeOH); cd MeOH $\Delta\epsilon$ (nm) 0 (356), −0.03 (335), +0.36 (290), +0.27 (270 sh), +0.44 (254); ^1H nmr (CD_3CN, 360 MHz) δ 5.00 (1H, m, H-22), 1.60 (3H, s, Me-27), 1.32 (3H, s, Me-18), 1.27 (3H, s, Me-19), 0.89 (3H, d, $J = 7.0$ Hz, Me-21); eims *m/z* $[M]^+$ 486 (10), 315 (11), 210 (13), 197 (12), 153 (21), 152 (26), 137 (36), 136 (65), 135 (18), 125 (100).

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LITERATURE CITED

1. R. Budhiraja and S. Sudhir, *J. Sci. Ind. Res.*, **46**, 488 (1987).
2. R. Tschesche, H. Schwang, and G. Legler, *Tetrahedron*, **22**, 1121 (1966).
3. R. Tschesche, M. Baumgarth, and P. Welzel, *Tetrahedron*, **24**, 5169 (1968).
4. R. Tschesche, K. Annen, and P. Welzel, *Chem. Ber.*, **104**, 3556 (1971).
5. D. Lavie, H. Gottlieb, M. Pestchanker, and O. Giordano, *Phytochemistry*, **25**, 1765 (1986).
6. E. Monteagudo, G. Burton, C. Gonzalez, J. Oberti, and E. Gros, *Phytochemistry*, **27**, 3925 (1988).
7. V. Fajardo, A. Freyer, R. Minard, and M. Shamma, *Tetrahedron*, **43**, 3875 (1987).
8. M. Parvez, V. Fajardo, and M. Shamma, *Acta Crystallogr.*, **C44**, 553 (1988).
9. D. Lavie, R. Bessalle, M. Pestchanker, H. Gottlieb, F. Frolow, and O. Giordano, *Phytochemistry*, **26**, 1791 (1987).
10. P. Demarco, E. Farkas, D. Doddrell, and B. Mylari, *J. Am. Chem. Soc.*, **90**, 5480 (1968).
11. D. Cookson and B. Smith, *Org. Magn. Reson.*, **16**, 111 (1981).

12. I. Kirson, D. Lavie, S. Subramanian, P. Sethi, and E. Glotter, *J. Chem. Soc., Perkin Trans. 1*, 2109 (1972).
13. G. Snatzke, *Angew. Chem. Int. Ed. Engl.*, **7**, 14 (1968).
14. E. Glotter, A. Abraham, G. Gunzberg, and I. Kirson, *J. Chem. Soc., Perkin Trans. 1*, 341 (1977).
15. F. Frolow, A. Ray, M. Sahai, E. Glotter, H. Gottlieb, and I. Kirson, *J. Chem. Soc., Perkin Trans. 1*, 1029 (1981).
16. A. Ray, M. Sahai, and B. Das, *J. Indian Chem. Soc.*, **55**, 1175 (1978).
17. A. Gonzalez, J. Breton, and J. Trujillo, *An. Quim.*, **70**, 69 (1974).
18. S. Nittala, V. Vande Velde, F. Frolow, and D. Lavie, *Phytochemistry*, **20**, 2547 (1981).
19. H. Gottlieb and I. Kirson, *Org. Magn. Reson.*, **16**, 20 (1981).
20. A.I. Scott, "Interpretation of the Ultraviolet Spectra of Natural Products," Pergamon Press, New York, 1964, p. 65.
21. D. Lavie, I. Kirson, E. Glotter, D. Rabinovich, and Z. Shakked, *Chem. Commun.*, 877 (1972).
22. H. Kessler, C. Griesinger, J. Zarbock, and H. Looslie, *J. Magn. Reson.*, **57**, 331 (1984).

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