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(+)-JABOROL, AN UNUSUAL PHENOLIC WITHANOLIDE FROM *JABOROSA MAGELLANICA*

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The B-secowithanolide (+)-jaborol (1) was obtained from Chilean Jaborosa magellanica (Solanaceae). It is probably the result of in vivo oxidative cleavage of the C-9(10) bond of a more classical withanolide precursor.

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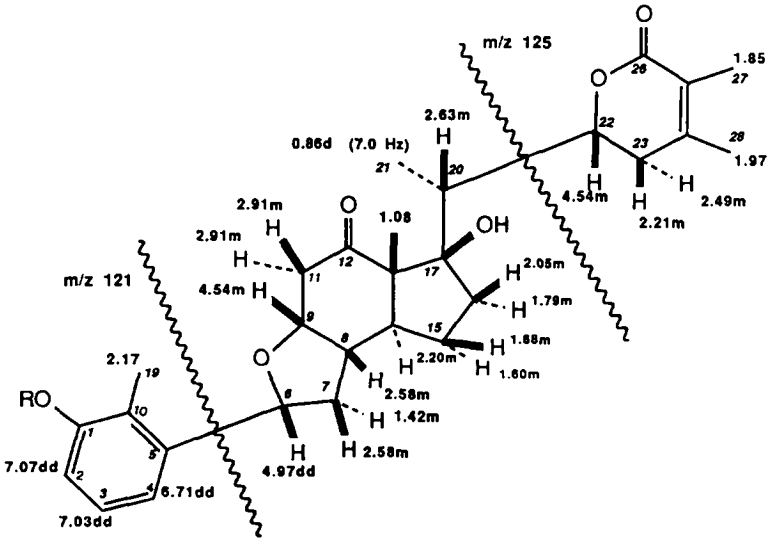
The withanolides, of which over 100 are presently known, are a group of C_{28} steroids found exclusively within the botanical family Solanaceae.[2-4] A ketonic function is usually present at C-1; while the C-17 side chain is made up of nine carbons, and includes either a γ- or a δ-lactone. The best known withanolide is withaferin A which is highly cytotoxic and has shown activity in several tumor systems.[5-8]

In the course of a systematic investigation of the withanolides of Jaborosa magellanica (Griseb.) Dusen (Solanaceae), collected in Chile along the northern shores of the Strait of Magellan, we isolated several new withanolides, some incorporating the γ-lactone side chain, and others the δ-lactone arrangement. All of these compounds were oxygenated at C-1, and also at C-12.[9]

The most interesting of our new compounds was the colorless, crystalline (+)-jaborol (1), $C_{28}H_{36}O_6$, whose spectral and chemical properties stood distinctly apart from those of the accompanying withanolides, or in fact from those of any of the other withanolides previously described in the literature.[2-4]



J_o = 7.6 Hz J_m = 1.5 Hz $J_{6,7\alpha}$ = 10.2 Hz $J_{6,7\beta}$ = 4.4 Hz

1, R = H (with NMR)

1a, R = Ac

One of the unusual features of (+)-jaborol (1) is that it is phenolic, and its UV spectrum shows a bathochromic shift in basic solution. Two IR carbonyl bands are in evidence, one at 1700 cm^{-1} due to the C-12 ketone, and the other at 1690 cm^{-1} representing the δ-lactone carbonyl.

The 360 MHz $CDCl_3$ NMR spectrum of (+)-jaborol has been summarized around expression 1. Noteworthy are the three sets of downfield doublet of doublets at δ 6.71, 7.03 and 7.07, representing the three contiguous aromatic protons of the phenolic ring. A three-proton singlet at δ 2.17 accounted for the benzylic methyl group. Another telling feature of the spectrum was the downfield one-proton doublet of doublets at δ 4.97 due to H-6. The presence of a ketonic function at C-12 is in line with all of the other withanolides of J. magellanica. The C-11 protons appeared as multiplets at δ 2.91 due to coupling with each other as well as with H-9 (δ 4.54).

The assignment of chemical shifts to the C-17 side chain is based on a number of relevant literature precedents.[10-12] The 17-hydroxyl must be beta since the 18-methyl absorption (δ 1.08) underwent a distinct downfield shift to δ 1.28 when the spectrum was run in pyridine-d_5.[12] Other proton absorptions that suffered downfield shifts in pyridine-d_5 are those for H-20 (from δ 2.63 to 3.24), and for H-22 (from δ 4.54 to 5.27), so that all of these hydrogens must be syn and also proximate to the 17-hydroxyl group.

The mass spectrum of (+)-jaborol (1) showed small molecular ion m/z 468 (3%), and significant peaks m/z 125 (44%) and 121 (34%) due to the cleavages indicated in expression 1. The base peak, m/z 109, represents a C_8H_{13} fragment originating from rings C and D of the molecule, as further confirmed by a high resolution mass spectral analysis.

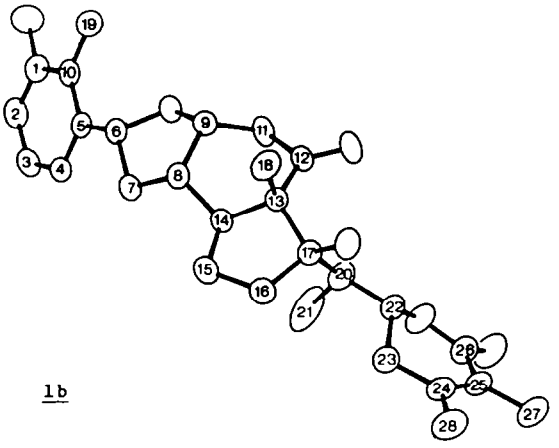
The CD curve of (+)-jaborol displayed a positive Cotton effect at 245 nm indicative of the 22R configuration as indicated in expression 1.[12,13]

Acetylation of (+)-jaborol using acetic anhydride in pyridine afforded (+)-jaborol acetate (1a), $C_{30}H_{38}O_7$, whose $CDCl_3$ NMR spectrum differed significantly from that of the parent withanolide only in the absorptions due to the protons related to ring A. In particular, the C-19 methyl singlet was located downfield at δ 2.33, while H-2, H-3 and H-4 were found at δ 7.44, 7.23 and 6.94, respectively.

The ^{13}C NMR spectrum of (+)-jaborol (1), supplemented by a GASPE analysis,[15] supplied useful structural data. Two carbonyl carbon absorptions were in evidence, one at δ 166.10 for C-26, and the other at 212.81 representing C-12. The 17-hydroxyl group must be beta since the C-21 signal is located relatively downfield at δ 13.04. If the hydroxyl in question had been alpha, the C-21 signal would have been found further upfield around δ 9.55.[16]

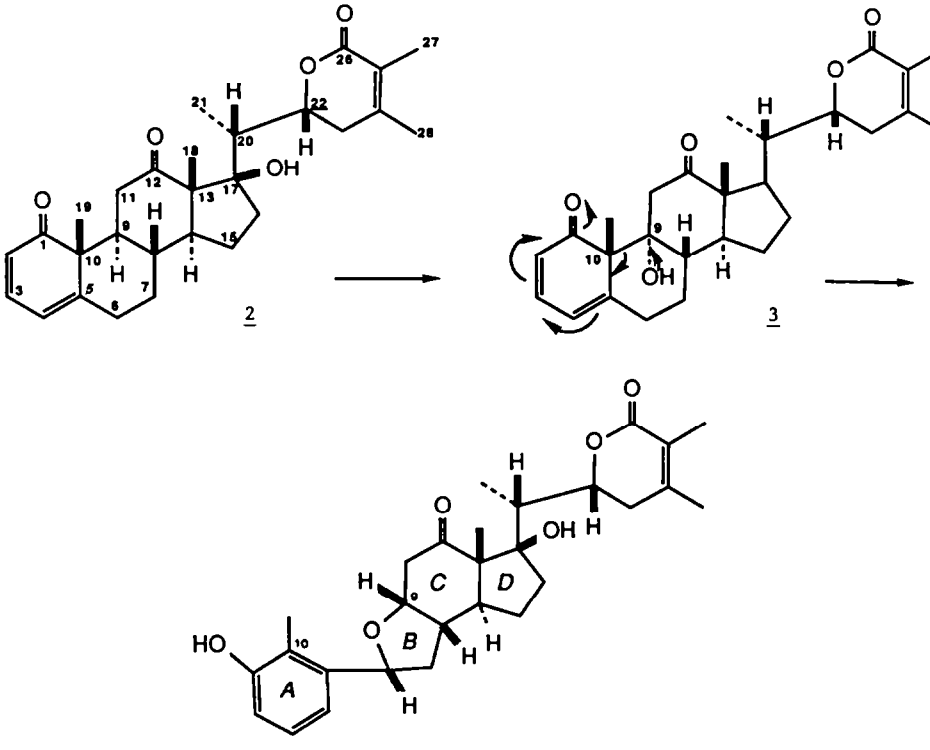
At this stage, we were interested in establishing the full stereochemistry of (+)-jaborol, particularly at C-6 and C-9. For this purpose, we had recourse to a complete NMR NOE analysis. Our initial point of reference was the 18-methyl group (δ 1.08), which generally in steroids stands above, i.e. beta, to the mean plane of the molecule. Irradiation of H-20 (δ 2.63) led to a 9.6% increase for the 18-methyl signal (δ 1.08), as well as to a 6.3% increase of H-22 (δ 4.54). Alternatively, irradiation of H-9 (δ 4.54) and H-11β (δ 2.91), effected increases of the 18-methyl signal of 6.6% and 5.8%, respectively. Another absorption that was affected by irradiation of H-9 (δ 4.54) was that for H-8 (δ 2.58) which underwent a 6.1% increment, so that the B/C fusion must be cis. Finally, irradiation of H-6 (δ 4.97) caused a 3.5% increase of H-9 (δ 4.54). The NOE results thus led to the stereochemistry for (+)-jaborol indicated in expression 1.

As final confirmation of the structure assignment, an x-ray analysis of (+)-jaborol was carried out.[17] The crystals are monoclinic, $P2_1$, a = 6.919(3), b = 17.042(3), c = 10.267(3) A°, β = 93.39(3)°, V = 1208.5 $A°^3$, Z = 2, D = 1.288 g cm^{-3}. The diffraction data led to expression 1c, shown below, for (+)-jaborol, which was in full agreement with structure 1 previously reached.



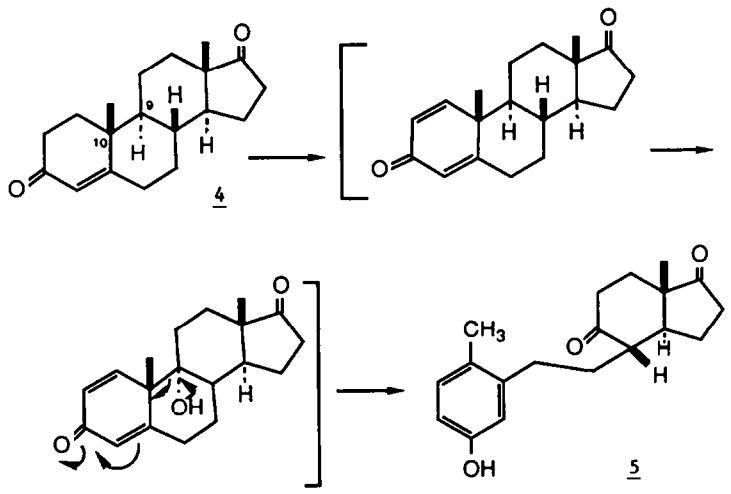
1b

The importance of (+)-jaborol (1) resides in the fact that it gives us an insight into the catabolism of the withanolides. Since these steroids usually bear a ketonic function at C-1, it is conceivable that the biogenetic precursor to 1 could be a species such as 2. In vivo oxidation of 2 at C-9 could lead to alcohol 3. Cleavage of the C-9 to C-10 bond, followed by the appropriate oxidation-reduction and dehydration would then lead to (+)-jaborol.



(+)-JABOROL

Interestingly enough, it was reported more than 25 years ago that incubation of 4-androstene-3,17-dione (4) with Pseudomonas or Arthrobacter species yielded 3-hydroxy-9,10-seco-1,3,5(10)-androstatriene-9,17-dione (5).[18] Evidently, these microorganisms as well as the plant J. magellanica possess related enzymes capable of achieving the oxidative cleavage of the C-9,10 bond of steroidal species, leading to B-seco compounds.



EXPERIMENTAL

The dried leaves and roots of J. magellanica (13.0 kg) were milled and extracted at room temperature with 95% ethanol. The extracts were concentrated under reduced pressure to afford a crude, oily mass (1.5 kg). This material was diluted with water and extracted with $CHCl_3$. Evaporation of the organic solvent provided a residue (30 g) of crude withanolides which were placed on a chromatographic column of Silica Gel 60 (70-230 mesh) (1 kg). Elution was with $CHCl_3$ containing increasing amounts of MeOH-EtOAc. Repeated TLC over silica gel using the system $CHCl_3$-MeOH (95:5) provided (+)-jaborol (110 mg), m.p. 135° C (MeOH). Under UV light, the TLC spot presented a blue coloration. When the developed TLC plate was sprayed with conc. H_2SO_4 and then heated over a hot surface for 2-3 min, a reddish-purple coloration appeared which was characteristic of this phenolic withanolide.

(+)-Jaborol (1):- ν max (KBr) 3400, 1700, 1690 cm^{-1}; λ max (MeOH) 224, 281, 292 nm (log ε 4.29, 3.51, 3.34); λ max (MeOH + OH^-) 224, 282, 301 nm (log ε 4.30, 3.51, 3.41); $[\alpha]_D$ +77.2° (c 0.40, MeOH); CD MeOH nm ($\Delta\varepsilon$) 300 (-1.3), 279 (0), 245 (+5.2). ^{1}H NMR in CD_3CN at 360 MHz: δ 0.82 (3H, d, J = 7.0 Hz, 21-Me), 0.99 (3H, s, 18-Me), 1.34 (1H, m, H-7α), 1.55 (1H, m, H-15α), 1.61 (2H, m, H-15β and H-16α), 1.79 (3H, s, 27-Me), 1.89 (3H, s, 28-Me), 2.05 (1H, m, H-16β), 2.11 (3H, s, 19-Me), 2.17 (1H, m, H-14), 2.23 (1H, m, H-23β), 2.44 (1H, br d, H-23α), 2.53 (3H, m, H-7α, H-8 and H-20), 2.73 (1H, dd, J_{gem} = 16.3 Hz, $J_{9,11\beta}$ = 5.9 Hz, H-11β), 2.89 (1H, dd, J_{gem} = 16.3 Hz, $J_{9,11\alpha}$ = 9.0 Hz, H-11α), 4.59 (1H, m, H-9), 4.65 (1H, dm, H-22), 4.94 (1H, dd, $J_{6,7\alpha}$ = 10.4 Hz, $J_{6,7\beta}$ = 4.5 Hz, H-6), 6.68 (1H, dd, J_o = 7.4 Hz, J_m = 1.3 Hz, H-4), 6.99 (1H, dd, J_o = 7.6 Hz, H-3), 7.03 (1H, dd, J_o = 7.6 Hz, J_m = 1.3 Hz, H-2). ^{1}H NMR in pyridine-d_5 at 360 MHz: δ 1.65 (3H, s,27-Me), 1.82 (3H, s,28-Me), 2.27 (2H, m, H-14 and H-23α), 2.43 (1H, br d, H-8), 2.51 (3H,

s. 19-Me), 2.56 (1H, m, H-7), 3.06 (1H, dd, $J_{9,11\beta}$ = 5.4 Hz, J_{gem} = 16.1 Hz, H-11β), 3.24 (3H, m, H-11α, H-20 and H-23β), 4.57 (1H, m, H-9), 5.12 (1H, dd, $J_{6,7\alpha}$ = 10.2 Hz, $J_{6,7\beta}$ = 5.0 Hz, H-6), 5.27 (1H, dm, H-22), 7.15 (1H, dd, J_o = 7.6 Hz, J_m = 1.3 Hz, H-4), 7.26 (1H, dd, J_o = 7.6 Hz, H-3), 7.44 (1H, dd, J_o = 7.6 Hz, J_m = 1.3 Hz, H-2).

NOE in $CDCl_3$ at 360 MHz: H-6β to H-7β (11.6%), H-6β to H-4 (3.0%), H-6β to H-9 (3.5%), H-11β to 18-Me (5.8%), H-11β to H-9 (7.6%), H-9 to H-8 (6.1%), H-9 to 18-Me (6.6%), H-11α to H-7α (3.0%), H-7α to H-14 (2.0%), H-8 to H-15β (8.3%), H-20 to 18-Me (9.6%), H-20 to H-22 (6.3%), H-23β to H-20 (8.1%).

^{13}C NMR in $CDCl_3$: Values with identical superscripts are interchangeable, δ 11.01 (C-19), 12.38 (C-27), 13.04 (C-21), 16.09 (C-18), 20.52 (C-28), 24.42 (C-15), 33.72 (C-23), 34.44 (C-16), 39.57 (C-7), 40.85 (C-20), 43.50* (C-8), 45.59 (C-13), 46.96* (C-14), 58.90 (C-11), 76.23# (C-9), 78.14# (C-6), 78.29# (C-22), 83.35 (C-17), 114.04¶ (C-2), 116.56¶ (C-4), 121.04§ (C-10), 121.67§ (C-25), 126.55 (C-3), 140.62 (C-5), 149.76 (C-1), 153.97 (C-24), 166.10 (C-26), 212.81 (C-12).

Low res. MS m/z 468 (M^+, 3), 453 (2), 450 (3), 424 (7), 319 (22), 315 (3), 241 (4), 153 (5), 152 (4), 151 (3), 150 (4), 147 (10), 136 (22), 135 (39), 125 (44), 121 (34), 109 (100), 107 (11).

High res. MS m/z 468.2460 ($C_{28}H_{36}O_6$), 453.2275 ($C_{27}H_{33}O_6$), 450.2397 ($C_{28}H_{34}O_5$, M - H_2O), 424.2593 ($C_{27}H_{36}O_4$, M - CO_2), 319.1923 ($C_{19}H_{27}O_4$), 315.1635 ($C_{19}H_{23}O_4$), 153.0940 ($C_9H_{13}O_2$), 147.0819 ($C_{10}H_{11}O$), 136.0885 ($C_9H_{12}O$), 135.0592 ($C_8H_7O_2$), 125.0617 ($C_7H_9O_2$), 121.0297 (C_8H_9O), 109.1017 (C_8H_{13}), 107.0498 (C_7H_7O).

(+)-Jaborol Acetate (1a):- m.p. 202° C (MeOH); ν max ($CHCl_3$) 1740, 1710, 1700 cm^{-1} (br bands); λ max (MeOH) 214, 233 sh nm (log ε 4.16, 3.84); $[\alpha]_D$ +164.7° (c 0.68, MeOH); CD MeOH nm (Δε) 300 (-0.04), 280 (0), 250 (+1.25). ^1H NMR in $CDCl_3$ at 360 MHz: δ 0.88 (3H, d, J = 7.0 Hz, 21-Me), 1.10 (3H, s, 18-Me), 1.42 (1H, m, H-7α), 1.94 (3H, s, 28-Me), 2.11 (3H, s, CH_3CO), 2.33 (3H, s, 19-Me), 2.49 (1H, br d, H-23α), 2.58 (2H, m, H-7β and H-8), 2.64 (1H, m, H-20), 2.93 (2H, m, H-11α and H-11β), 4.51 (1H, m, H-22), 4.55 (1H, m, H-9), 4.98 (1H, dd, $J_{6,7\alpha}$ = 9.8 Hz, H-6), 6.94 (1H, dd, J_o = 8.0 Hz, J_m = 1.5 Hz, H-4), 7.23 (1H, dd, J_o = 7.8 Hz, H-3), 7.44 (1H, dd, J_o = 7.8 Hz, J_m = 1.5 Hz, H-2).

Low res. MS m/z 510 (M^+,1), 492 (3), 474 (4), 451 (1), 357 (7), 319 (20), 297 (6), 283 (6), 241 (10), 177 (13), 165 (4), 163 (12), 153 (12), 149 (6), 147 (17), 137 (37), 136 (45), 135 (39), 125 (100), 121 (33), 109 (50), 107 (14), 97 (30).

High res. MS m/z 492.2467 ($C_{30}H_{36}O_6$, M - H_2O), 451.2520 ($C_{28}H_{35}O_5$, M - acetate), 357.1720 ($C_{21}H_{25}O_5$), 319.1923 ($C_{19}H_{27}O_4$), 297.1494 ($C_{19}H_{21}O_3$), 197.1030 ($C_{10}H_{13}O_4$), 153.0886 ($C_9H_{13}O_2$), 147.0886 ($C_9H_{13}O_2$), 147.0809 ($C_{10}H_{11}O$), 136.0876 ($C_9H_{12}O$), 135.0448 ($C_8H_7O_2$), 125.0609 ($C_7H_9O_2$), 121.0658 (C_8H_9O), 109.1014 (C_8H_{13}).

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REFERENCES AND FOOTNOTES

1. Permanent address: Universidad de Magallanes, Punta Arenas, Chile.
2. R.N. Tursunova, V.A. Maslennikova and N.K. Abubakirov, Khim. Prir. Soedin., 147, (1977); Chem. Nat. Cpds., 131 (1977).
3. A.V. Kamernitskii, I.G. Reshetova and V.A. Krivoruchko, Khim. Prir. Soedin., 156 (1977); Chem. Nat. Cpds., 138 (1977).
4. O.E. Vasina, V.A. Maslennikova and N.K. Abubakirov, Khim. Prir. Soedin., 263 (1986); Chem. Nat. Cpds., 243 (1986).
5. S.M. Kupchan, R.W. Doskotch, P. Bollinger, A.T. McPhail, G.A. Sim and J.A.S. Renauld, J. Am. Chem. Soc., 87, 4505 (1965).
6. B. Shohat, S. Gitter, A. Abramson and D. Lavie, Cancer Chemother. Rep., 51, 271 (1967).
7. I. Palyi, E. Tyihak and V. Palyi, Herba Hung., 8, 73 (1969).
8. B. Shohat, S. Gitter and D. Lavie, Int. J. Cancer, 5, 244 (1970).
9. These new withanolides from J. magellanica will be reported in separate publications.
10. D. Lavie, I. Kirson and E. Glotter, Chem. Commun., 877 (1972).
11. E. Glotter, A. Abraham, G. Günzberg and J. Kirson, J. Chem. Soc., Perkin I, 341 (1977).
12. V. Velde and D. Lavie, Phytochem., 20, 1359 (1981).
13. I. Kirson, D. Lavie, S.S. Subramanian, P.D. Sethi and E. Glotter, J. Chem. Soc., Perkin I, 2109 (1972).
14. G. Snatzke, Angew. Chem. Internat. Edn., 7, 14 (1968).
15. D.J. Cookson and B.E. Smith, Org. Magn. Reson., 16, 111 (1981).
16. H. Gottlieb and I.Kirson, Org. Magn. Reson., 16, 20 (1981).
17. The x-ray analysis was carried out by Dr. Masood Parvez of the Department of Chemistry, PSU, and will be reported in full elsewhere.
18. R.M. Dodson and R.D. Muir, J.Am. Chem. Soc., 83, 4627 (1961).