Two New Unusual Diterpenoids from *Ballota hispanica*

Benjamín Rodríguez,[1a] Giuseppe Savona,[1b] and Franco Piozzi*[1b]

Instituto de Química Orgánica General, C.S.I.C., Madrid-6, Spain,
and Institute of Organic Chemistry, University of Palermo, Palermo, Italy

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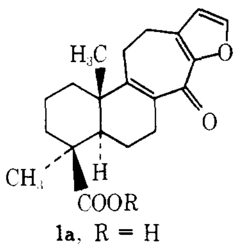
From the acetone extract of *B. hispanica* hispanonic acid (**1a**, $C_{20}H_{24}O_4$) was isolated; CH_2N_2 treatment of other chromatographic fractions and extended purification yielded methyl hispanonate **1b** and methyl hispaninate **6b** ($C_{21}H_{26}O_4$). Both products have an unusual seven-membered ring C. DDQ aromatization and catalytic hydrogenation of **1b** are described; **6b** gave a crystalline peroxide, confirming the proposed structures. Correlation of **1b** and **6b** was achieved through reduction products.



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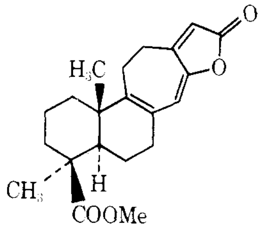
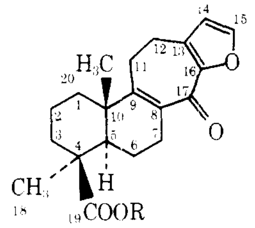


6b

Continuing our studies on medicinal plants of the Mediterranean area, some members of the genus *Ballota* (Labiatae) have been recently investigated.[2] From the acetone extract of the species *Ballota hispanica* Neck. ex Nim. (synonymous *B. hirsuta* Benth.) growing in Spain, we isolated the labdanic furanoditerpene hispanolone[3] and two novel diterpenes, hispanonic and hispaninic acids, whose unusual structures are reported here.



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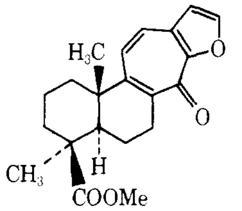

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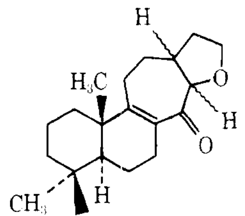

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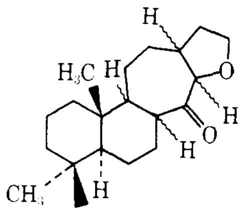

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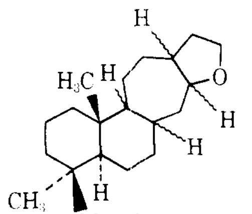

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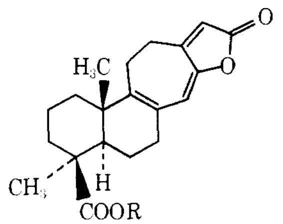

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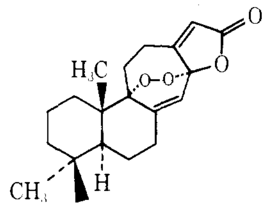

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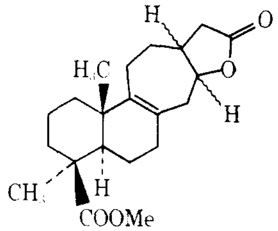

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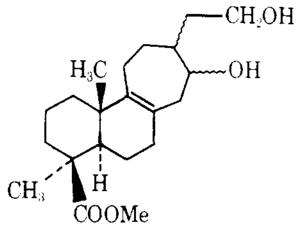

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Hispanonic acid (**1a**, $C_{20}H_{24}O_4$) was isolated as a free acid and as its methyl ester (**1b**, $C_{21}H_{26}O_4$) after diazomethane treatment of some fractions arising from extensive chromatography of the plant extract. The IR spectrum of compound **1b** showed bands for an ester group (1720 cm^{-1}) and a furan ring (880 cm^{-1}), whereas a strong carbonyl absorption at 1605 cm^{-1} was attributed to an α,β-unsaturated ketone which was also conjugated to the furan ring. In agreement with this hypothesis, the UV spectrum of **1b** showed multiple absorptions at λ_{max} 313, 284, 273, and 258 nm. On the other hand, the ^1H NMR spectrum of compound **1b** exhibited characteristic signals for two tertiary methyl groups (δ 1.19 and 0.88), one carbomethoxyl group (3 H, singlet at δ 3.60), and only two furan protons at δ 6.26 (doublet, J = 2 Hz, β-furan proton) and 7.42 (doublet, J = 2 Hz, α-furan proton). These data and the empirical formula of compound **1b** indicated the presence of a furan ring α,β fused to a tricyclic hydrocarbon skeleton containing an additional tetrasubstituted olefinic double bond, a highly conjugated ketone, and also a carbomethoxyl group.

As compound **1b** possessed only three terminal carbon atoms (two methyl and one carbomethoxyl groups), a seven-membered ring was likely to be present, and structure **1a** was assigned to hispanonic acid by the following reasons.

The ^{13}C NMR spectrum of compound **1b** showed charac-

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Table I. ^{13}C NMR Spectra of Compounds 1b, 6b, and 7[a]

	1b	**6b**	**7**
C-1	37.4 t	37.4 t	32.0 t
C-2	19.5 t	19.5 t	18.9 t
C-3	36.4 t	36.7 t	37.6 t
C-4	43.9 s	43.8 s	42.6 s
C-5	53.1 d	53.0 d	43.2 d
C-6	20.2 t	20.7 t	21.1 t
C-7	28.2 t	34.1 t	26.8 t
C-8	134.8 s	126.1 s	150.4 s
C-9	150.5 s	147.7 s	88.6 s
C-10	41.4 s	40.8 s	45.3 s
C-11	25.1 t	25.0 t	24.9 t
C-12	27.7 t	27.0 t	22.3 t
C-13	135.7 s	151.1 s	168.0 s
C-14	112.7 d	116.3 d	119.0 d
C-15	145.1 d	169.1 s	170.0 s
C-16	154.0 s	157.9 s	104. 6 s
C-17	183.1 s	113.6 d	111.7 d
C-18	28.2 q	28.3 q	28.3 q
C-19	177.7 s	177.4 s	177.4 s
C-20	16.7 q	16.9 q	20.4 q
OMe	51.1 q	51.1 q	51.3 q

[a] δ_C in parts per million from Me_4Si.

teristic carbon resonances for an α,β-α',β'-diunsaturated ketone (singlet at 183.1 ppm), a methyl ester group (singlet at 177.7 ppm and a quartet at 51.1 ppm), and six olefinic carbon atoms (four singlets and two doublets), corresponding to an α,β-fused furan ring and a tetrasubstituted olefinic double bond (see Table I). On the other hand, the C-1, C-2, C-3, C-4, C-5, C-6, C-8, C-9, C-10, C-18, and C-19 carbon resonances were in good agreement with previously reported values for the carbon atoms in rings A and B of diterpenoids containing an aromatic C ring and an axial C-19 carbomethoxyl group.[4] The upfield shift ($\Delta\delta = -6.3$ ppm) of the C-20 resonance and the small variations in the C-1 and C-10 resonances (see ref 4 and Table I) reflected the structural difference in C-11 and C-12 between aromatic diterpenoids and compound **1b.** The C-7 resonance showed an upfield shift comparing **1b** with aromatic diterpenoids ($\Delta\delta = -3.2$ ppm), and this must be only caused by a shielding γ effect of the carbonyl group,[5] which must be placed on C-17.

Treatment of compound **1b** with dichlorodicyanoquinone (DDQ) gave the dehydro derivative **2** ($C_{21}H_{24}O_4$), whose ^1H NMR spectrum showed two more ethylenic protons as an AB quartet ($J = 12.5$ Hz) at δ 7.02 and 7.18, identical with those previously reported for similar tropolone derivatives.[6] Its more complex UV spectrum indicated the presence of an aromatic system.

Catalytic hydrogenation of **1b** over Pd/C at 60 °C yielded three derivatives: the tetrahydrofuran **3** ($C_{21}H_{30}O_4$), the hexahydro derivative **4** ($C_{21}H_{32}O_4$), and the octahydrodeoxo derivative **5** ($C_{21}H_{34}O_3$). Characteristic features of **3** were the α,β-conjugated carbonyl absorption at 252 nm, the signals of the C-15 methylene at δ 3.90 and 4.25, and the sharp doublet ($J = 9.5$ Hz) of the H-16 proton at δ 4.65. Products **4** and **5** lacked any UV absorption; in the case of compound **5**, the empirical formula and the upfield shift of the H-16 to δ 3.85 (now a multiplet) excluded the occurrence of the ketone, which was also proved because **5** was recovered unchanged after $NaBH_4$ treatment under normal conditions.

The carbomethoxyl group of compound **1b** was placed on C-4 and proved to be axial (besides the ^{13}C NMR reasons mentioned above) on the basis of the following evidence. The IR spectra of **4** and **5** showed absorption at 1150 and 1155 cm^{-1}, respectively, typical of an axial methyl ester function,[7] whereas in the ^1H NMR spectrum of compound **1b** irradiation of the COOMe signal gave a clear Overhauser effect on the 20-Me signal and vice versa.

The other new diterpenoid isolated from *B. hispanica,* hispaninic acid (**6a**), was obtained as its methyl ester (**6b,** $C_{21}H_{26}O_4$) after diazomethane treatment of some fractions of the extract and subsequent extensive chromatography. The four oxygen atoms were attributed to the ester group and to a conjugated α,β-unsaturated γ-lactone, according to the occurrence of absorption bands at 1720 and 1735 cm^{-1} in its IR spectrum. An extended conjugation was shown by the UV spectrum (λ_{max} 357, 260, and 233 nm), and the ^1H NMR spectrum indicated the presence of two tertiary methyl groups (δ 1.23 and 0.84) and a carbomethoxyl group (δ 3.62), as in compound **1b,** and of two long range coupled ethylenic protons at δ 5.89 and 5.76 instead of the furan protons of compound **1b.**

Exposure of an alcoholic solution of **6b** to air and visible light, in the presence of Bengal Rose B,[8] yielded a crystalline peroxide (**7**, $C_{21}H_{26}O_6$) whose α,β-unsaturated γ-lactone lacked the previously existing extended conjugation, as revealed by its UV (λ_{max} 222 nm) and IR (ν_{max} 1770 cm^{-1}) spectra.

The spectral data (^1H NMR, UV, and IR) of **6b** did not rule out other isomeric structures; moreover, alternative structures (i.e., γ-lactone ring closure on C-12) were excluded by comparison of the ^{13}C NMR spectra of **6b** and **7** (see Table I). Indeed, a strong γ effect ($\Delta\delta = -9.8$ ppm) for the doublet of C-5 appeared in the spectrum of **7** with respect to **6b,** thus indicating the position of the peroxide bridge between C-9 and C-16, which was also confirmed by the C-20 chemical shift value[9] and the γ effects observed on C-1 and C-12 ($\Delta\delta = -5.4$ and -4.7 ppm, respectively). The structures of **6b** and **7** were therefore proved.

Reduction of **6b** with methanolic $NaBH_4$ at 60 °C gave two products, the tetrahydro lactone **8** and the ring-opened diol **9**. The first substance had typical IR absorptions for a saturated γ-lactone (1760 cm^{-1}) and an ester group (1720 cm^{-1}), and it had a multiplet at δ 4.48 for H-16 in its NMR spectrum. The second substance showed IR bands for ester (1720 cm^{-1}) and hydroxyl groups (3330 and 3240 cm^{-1}) and NMR multiplets at δ 4.22 for H-16 and at δ 3.76 for the C-15 methylene.

A correlation between hispaninic and hispanonic acids was achieved as follows. Diol **9**, obtained from methyl hispaninate **6b,** was hydrogenated on Pd/C; then, the resulting saturated diol was treated with tosyl chloride and pyridine to yield a product identical in all respects with the octahydrodeoxo derivative **5** prepared by catalytic hydrogenation of hispanonate **1b.** Thus, the structure of **1b** was also confirmed.

The absolute configuration of these new diterpenoids was not ascertained; however, **1b** and **6b** are believed to belong to the *normal* series like hispanolone,[3] co-occurring in the same species.

Seven-membered ring C is a very rare feature in tricyclic diterpenes; the only previously known cases are those of strobic acid and the related strobal and strobol from the resin of *Pinus strobus.*[10-12] The hydrocarbon skeleton of these last substances is not, however, the same as that of hispaninic and hispanonic acids.

Experimental Section

All melting points were determined in Kofler apparatus and are uncorrected. The optical rotations were measured with a Perkin-Elmer 141 polarimeter. UV spectra were determined on a Perkin-Elmer 402 recording spectrophotometer and IR spectra on a Perkin-Elmer 257 spectrometer. The ^1H NMR spectra were recorded on a 100-MHz Varian XL-100 apparatus and the ^{13}C NMR spectra on a 20- or 25.2-MHz Varian FT-80 or FT XL-100 instrument, all in $CDCl_3$ solution with tetramethylsilane as an internal standard. The mass spectra were obtained on a Jeol J-MS-01SG-2 instrument or on a Hitachi Perkin-Elmer RMU-6MG apparatus. Thin-layer chromatography was carried out on commercially prepared TLC plates (Merck). Column chromatography was carried out on silica gel (Kieselgel 60, 70–230 mesh, ASTM) supplied by Merck.

Extraction of *Ballota hispanica*. Flowers and leaves were collected near Hellin (Albacete, Spain) in June 1977. Air-dried and powdered material (2.3 kg) was extracted with acetone (12 L) at room temperature for 1 week. Solvent was evaporated, the residue extracted with ethyl acetate, and the extract washed with water and dried. Solvent was evaporated to give a gum which was subjected to dry column chromatography over silica gel (deactivated with 15% water). Elution with light petroleum gave plant waxes which were rejected. Elution with 9:1 light petroleum–ethyl acetate yielded hispanolone[3] (5.5 g); elution with 3:2 light petroleum–ethyl acetate gave a mixture of hispanonic and hispaninic acids, from which only a small quantity of hispanonic acid **1a** (20 mg) could be isolated. The mixture was methylated with an ethereal solution of diazomethane and subjected to extensive chromatography on silica gel, 4:1 light petroleum–ethyl acetate eluent, to give methyl hispaninate **6b** (50 mg) and methyl hispanonate **1b** (200 mg).

Hispanonic acid (**1a**) had mp 235 °C (from EtOAc); $[\alpha]^{20}{}_{D}$ +258° (*c* 0.46, $CHCl_3$); UV (EtOH) λ_{max} nm (ϵ) 311 (10 800), 281 sh (8500), 273 (8900), 254 sh (5900); IR (KBr) 3300–2500, 1720 broad, 1605 cm^{-1}; MS *m/e* 328 (M^+), 313, 285, 267, 213, 150. Anal. Calcd for $C_{20}H_{24}O_4$: C, 73.14; H, 7.37. Found: C, 72.88; H, 7.60.

Methyl hispanonate (**1b**) had mp 210–211 °C (EtOAc–petroleum ether); $[\alpha]^{22}{}_{D}$ +257° (*c* 0.59, $CHCl_3$); UV (EtOH) λ_{max} nm (ϵ) 313 (11 500), 284 sh (8800), 273 (9300), 258 sh (7000); IR (KBr) 1720, 1605, 880 cm^{-1}; MS *m/e* 342 (M^+), 327, 295, 283, 267, 213, 201, 185, 175, 150; ^{1}H NMR δ 7.42 (1 H, d J = 2 Hz, H-15), 6.26 (1 H, d, J = 2 Hz, H-14), 3.60 (3 H, s, COOMe), 1.19 and 0.88 (3 H each, s, H-18 and H-20 protons, respectively); Overhauser effect, irradiation of the signal at δ 0.88 causes an NOE of 19% on the δ 3.60 signal and vice versa; ^{13}C NMR, see Table I. Anal. Calcd for $C_{21}H_{26}O_4$: C, 73.66; H, 7.66. Found: C, 73.64; H, 7.76.

Methyl hispaninate (**6b**) had mp 166 °C (EtOAc–petroleum ether); $[\alpha]_D{}^{24}$ +387° (*c* 0.31, $CHCl_3$); UV (EtOH) δ_{max} nm (ϵ) 357 (10 400), 260 (6100), 233 (5300); IR (KBr) 1735, 1720, 1600 cm^{-1}; MS *m/e* 342 (M^+), 327, 310, 282, 267, 213, 174, 107; ^{1}H NMR δ 5.89 (1 H, m, $W_{1/2}$ 3 Hz, H-14), 5.76 (1 H, m, $W_{1/2}$ = 4 Hz, H-17), 3.62 (3 H, s, COOMe), 1.23 and 0.84 (3 H each, s, H-18 and H-20 protons, respectively); ^{13}C NMR, see Table I. Anal. Calcd for $C_{21}H_{26}O_4$: C, 73.66; H, 7.66. Found: C, 73.82; H, 7.60.

Dehydrogenation of 1b. Methyl hispanonate (**1b,** 100 mg) and DDQ (130 mg) were mixed with dioxane (10 mL) previously saturated with gaseious HCl and left at 20 °C for 24 h. After an aqueous solution of $NaHCO_3$ was added, the organic layer was extracted with EtOAc. Evaporation of the solvent at reduced pressure gave a residue which was chromatographed on silica gel with 7:3 petroleum ether–EtOAc. Crystallization from the same solvent yielded **2** (70 mg): mp 220 °C; $[\alpha]_D{}^{23}$ +338° (*c* 0.12, $CHCl_3$); UV (EtOH) λ_{max} nm (ϵ) 346 (2500), 331 (3600), 319 (3700), 308 sh (3400), 258 (17 700), 233 sh (10 300); IR (KBr) 1710, 1565, 890 cm^{-1}; MS *m/e* 340 (M^+), 325, 281, 265, 225, 211, 199, 185, 160; ^{1}H NMR δ 7.70 (1 H, d, J = 2 Hz, H-15), 7.18 and 7.02 (1 H each, AB system, J = 12.5 Hz, H-11 and H-12), 6.67 (1 H, d, J = 2 Hz, H-14), 3.62 (3 H, s, COOMe), 1.24 and 1.11 (3 H each, s, H-18 and H-20 protons, respectively). Anal. Calcd for $C_{21}H_{24}O_4$: C, 74.09; H, 7.11. Found: C, 73.93; H, 7.05.

Catalytic Hydrogenation of 1b. Methyl hispanonate (**1b,** 200 mg) dissolved in EtOAc (30 mL) was hydrogenated over 10% Pd/C (150 mg) at 60 °C for 1.5 h and then at 20 °C for 4 h. The catalyst was filtered and the solvent evaporated at reduced pressure; the resulting oil was chromatographed on silica gel, 7:3 petroleum ether–EtOAc eluent, to give **5** (40 mg), **3** (25 mg), and **4** (100 mg).

The octahydrodeoxo derivative **5**, $C_{21}H_{34}O_3$, had mp 110–112 °C (petroleum ether–EtOAc); IR (KBr) 1725, 1155 cm^{-1}; UV, no absorption; MS *m/e* 334 (M^+), 319, 302, 290, 275, 257, 221, 205, 180, 161, 149, 137, 121; ^{1}H NMR δ 3.85 (3 H, m, $W_{1/2}$ = 18 Hz, H-16 and 2H-15 protons), 3.68 (3 H, COOMe), 1.19 and 0.80 (3 H each, s, H-18 and H-20 protons, respectively).

The tetrahydrofuran **3,** $C_{21}H_{30}O_4$, had mp 195 °C (petroleum ether–EtOAc); $[\alpha]^{20}{}_{D}$ +258° (*c* 0.60, $CHCl_3$); UV (EtOH) λ_{max} nm (ϵ) 252 (7300); IR (KBr) 1720 and 1675 cm^{-1}; MS *m/e* 346 (M^+), 331, 318, 258, 243, 137, 121; ^{1}H NMR δ 4.65 (1 H, d, J = 9.5 Hz, H-16), 4.25 and 3.90 (2 H, m, H-15 protons), 3.62 (3 H, s, COOMe), 1.21 and 0.86 (3 H each, s, H-18 and H-20 protons, respectively).

The hexahydro derivative **4,** $C_{21}H_{32}O_4$, had mp 126–128 °C (petroleum ether–EtOAc); $[\alpha]^{27}{}_{D}$ +22° (*c* 0.10, $CHCl_3$); UV, no absorption; IR (KBr) 1720 and 1150 cm^{-1}; MS *m/e* 348 (M^+), 333, 320, 290, 260, 245, 221, 191, 161, 137, 121; ^{1}H NMR δ 4.30 (1 H, d, J = 9.5 Hz, H-16), 3.89 (2 H, m, H-15 protons), 3.69 (3 H, s, COOMe), 1.13 and 0.69 (3 H each, s, H-18 and H-20 protons, respectively). Anal. Calcd for $C_{21}H_{32}O_4$: C, 72.38; H, 9.26. Found: C, 72.43; H, 9.17.

Photooxidation of 6b. Peroxide 7. A solution of methyl hispaninate (**6b,** 20 mg) in ethanol (50 mL) containing traces of Bengal Rose B was kept under an O_2 atmosphere and exposed to sunlight during 24 h. After the solvent was evaporated, the residue was chromatographed on silica gel with 1:1 petroleum ether–EtOAc to give 15 mg of the peroxide **7**: mp 148–150 °C (petroleum ether–EtOAc); $[\alpha]^{24}{}_{D}$ +82° (*c* 0.25, $CHCl_3$); UV (EtOH) λ_{max} 222 nm (ϵ 15 900); IR (KBr) 1770 and 1715 cm^{-1}; MS *m/e* 374 (M^+), 359, 356, 342, 314, 282, 234, 182, 161, 147, 123; ^{1}H NMR δ 5.92 (1 H, m, allylic coupling, $W_{1/2}$ = 3 Hz, H-14), 5.62 (1 H, m, allylic coupling, $W_{1/2}$ = 3.5 Hz, H-17), 3.64 (3 H, s, COOMe), 1.20 and 1.16 (3 H each, s, H-18 and H-20 protons, respectively); ^{13}C NMR, see Table I. Anal. Calcd for $C_{21}H_{26}O_6$: C, 67.36; H, 7.00. Found: C, 68.05; H, 6.99.

Sodium Borohydride Reduction of 6b. Tetrahydro Derivative 8 and Diol 9. An excess of $NaBH_4$ was slowly added (3 h) to a solution of compound **6b** (50 mg) in methanol (10 mL) at 60 °C. After the usual workup, column chromatography (7:3 petroleum ether–EtOAc) gave 8 (13 mg) and **9** (31 mg).

Product **8,** $C_{21}H_{30}O_4$, had mp 192 °C (hexane–EtOAc); $[\alpha]^{20}{}_{D}$ +136° (*c* 0.45, $CHCl_3$); UV, no absorption; IR (KBr) 1760 and 1720 cm^{-1}; MS *m/e* 346 (M^+), 314, 287, 271, 253, 201, 173, 159, 131; ^{1}H NMR δ 4.48 (1 H, m; $W_{1/2}$ = 20 Hz, H-16), 3.62 (3 H, s, COOMe), 1.18 and 0.76 (3 H each, s, H-18 and H-20 protons, respectively).

Product **9**, $C_{21}H_{34}O_4$, was an oil: UV, no absorption; IR (NaCl) 3330, 3240, 1720 cm^{-1}; MS *m/e* 350 (M^+), 317, 288, 273, 257, 239, 185, 159, 131; ^{1}H NMR δ 4.22 (1 H, m $W_{1/2}$ 18 Hz, H-16), 3.76 (2 H, m, $W_{1/2}$ = 12 Hz, H-15 protons), 3.65 (3 H, s, COOMe), 1.20 and 0.75 (3 H each, s, H-18 and H-20 protons, respectively).

Transformation of the Diol 9 into the Octahydrodeoxy Derivative 5. A solution of diol **9** (30 mg) in EtOAc (30 mL) was heated at 60 °C and hydrogenated over 10% Pd/C for 36 h. The catalyst was filtered and the solvent evaporated,; the oily residue (homogeneous on TLC, $AgNO_3$–silica gel) was allowed to stand overnight at room temperature in a solution of tosyl chloride (0.1 g) in pyridine (3 mL). Chromatography on silica afforded the octahydrodeoxo derivative 5, which was identified by its spectral data (IR, ^{1}H NMR, and MS).

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Registry No.—**1a,** 69912-64-7; **1b,** 69912-65-8; **2,** 69912-66-9; **3,** 69912-67-0; **4,** 69912-68-1; **5,** 69912-69-2; **6a,** 69912-70-5; **6b,** 69912-71-6; **7,** 69912-72-7; **8,** 69912-73-8; **9,** 69912-74-9; hispanolone, 18676-07-8.

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