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Synthesis of 2-Oxygenated Glycyrrhetic Acid Derivatives

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2β,3α-Dihydroxy and 2β,3β-dihydroxy derivatives of glycyrrhetic acid and 11-deoxoglycyrrhetic acid were synthesized. Unsaturation was introduced at C-18 in both the 2β,3α and in the 2β,3β series of glycyrrhetic acid. Some of the compounds exhibited low levels of antibacterial and antifungal activity.

Studies in this laboratory to achieve a separation of activities for derivatives of glycyrrhetic acid have focused on rings A and E.[1] Since the mammalian system metabolizes many triterpenes by oxygenation,[1] it was felt that a synthesis of some oxygenated derivatives of glycyrrhetic acid might yield products with a different spectrum of activities from the parent compound. This paper reports oxygenations at positions 2 and 3 of the oleane skeleton, as well as the introduction of unsaturation at C-18.

2β,3α-Dihydroxy Derivatives. Mousseron-Canet and Crouzet[2] reported the synthesis of **1a** and **1b**. Hydrogenolysis of this mixture with PtO_2 in acetic acid yielded a mixture of the corresponding 11-deoxo compounds **2** and **3**, which were separated by column chromatography. The dihydroxy compound **4** could be derived from a mixture of **2** and **3** by refluxing in methanolic KOH. The C-30 ester is sufficiently hindered so that no saponification occurs at this site. The 11-oxo analog of **4**, compound **5**, was made by direct treatment of a mixture of **1a** and **1b** with refluxing methanolic KOH. Treatment of the C-30 ester to yield the free acid **6** was effected with LiI in refluxing 2,4,6-collidine.

Unsaturation at C-18 was introduced by the method of Ruzicka and Jeger.[3] Thus, acetylation of a mixture of **1a** and **1b** yielded the 2β,3α-diacetoxy compound, which was treated with Br_2 in hot glacial acetic acid in the presence of HBr. Separation by column chromatography yielded **7**, which was completely hydrolyzed to the free acid **8** with refluxing methanolic KOH (Scheme I).

2β,3β-Dihydroxy Substitutions. Treatment of **9b**[1] with *t*-BuOK and O_2 in *t*-BuOH and hexamethylphosphoric triamide by the method of Hanna and Ourisson[4] yielded the Δ^1-2-hydroxy-3-oxo compound **10b**. Reduction of **10b** with $NaBH_4$ yielded the 2β,3β-dihydroxy compound **12b**. Reaction of **10b** with Br_2 in THF with an HBr catalyst yielded **11**. In the 11-oxo series, **10a** was synthesized from **9a**[5] in the same manner as **10b** was derived from **9b**. The preparation of **12a** from **10a** was performed with $NaBH_4$ at 0–12° for 75 min. Acetylation of **12a** yielded **13**, which was converted to the Δ^{18} compound **14** (*vide supra*) (Scheme II).

An alternative method of *cis* hydroxylation involved reaction of the Δ^2 compound **15**[2] with OsO_4. Hydroxylation followed by crystallization resulted in the isolation of only the 2α,3α isomer **16**. When the products of hydroxylation were hydrogenolyzed and then separated by chromatography, isomers **17** and **18** were isolated (Scheme III).

Biology. All compounds were tested for anti-DCA activity by the method of Kagawa.[6] The test drug was injected subcutaneously at a dose of 2.4 mg/rat in a series of eight rats per dose. The standard drug in this assay is spironolactone, which displays a median effective dose of 0.33 mg/rat in this test. None of the compounds showed activity in this test.

Many of the compounds were tested for antiulcer activity in the pylorus ligated Shay rat,[7] tested in groups of six. Only **13** showed activity (at 50 mg/rat), as it did in the Shay rat antisecretory test,[8] in which it caused a 29% reduction in acid secreted in a 5-hr period. A group of six rats received 25 mg/rat in this test.

A number of compounds were tested for antiviral activity against influenza A (strain 575). The test compound was added to cell cultures of primary rhesus monkey kidney 1 hr before challenge with virus. The quantitative hemagglutination technique of Finter[9] was then used to assay activity. Compounds **6** and **13** were active at 625 μg/ml, but their cytotoxicity precluded their pursuit as leads.

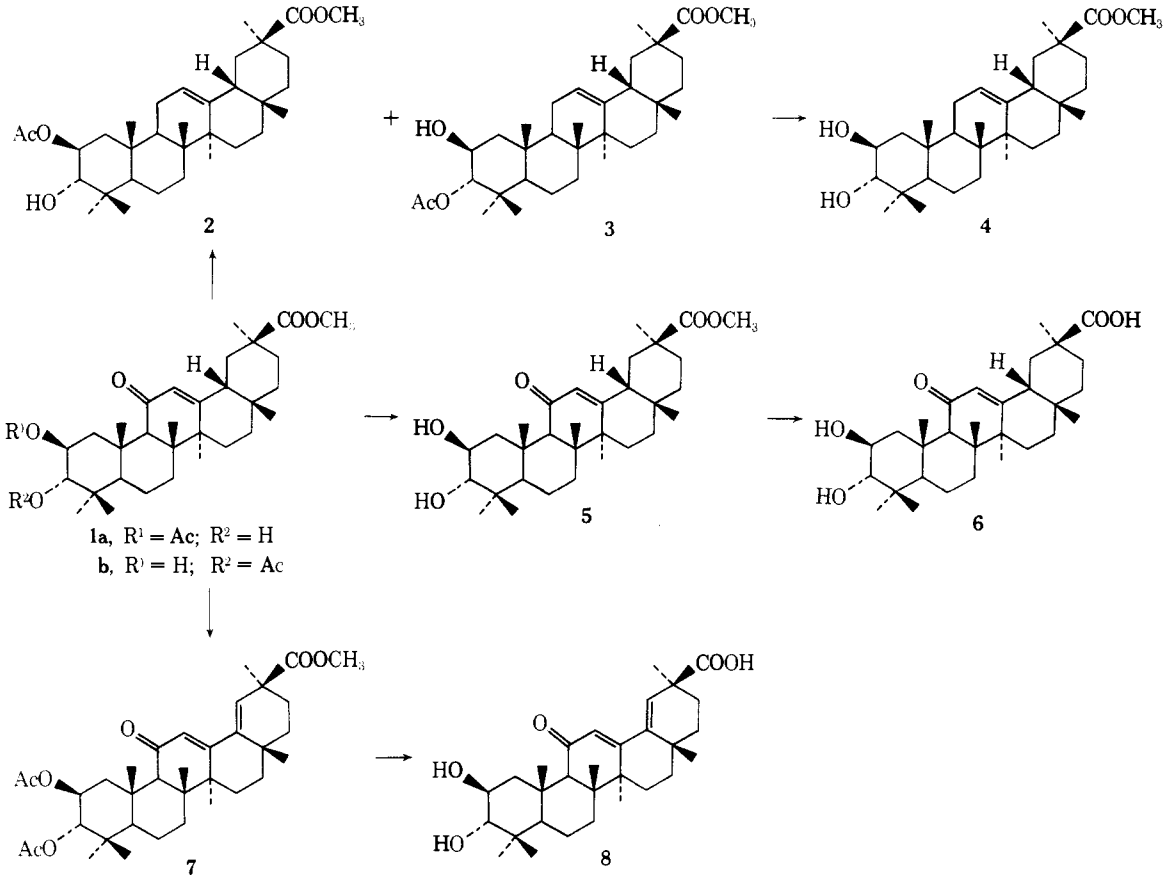
Some of these compounds showed weak *in vitro* antibacterial and antifungal activity. **8**, **12a**, **13**, and **14** were active at 100 ppm against *Erwinia* species in a beef extract growth medium, and **4**, **6**, and **11** were active at 1000 ppm against the same bacteria. **6** and **14** were active against *Bacillus subtilis* in beef extract at 1000 ppm. **11** was active against *Trichophyton mentagrophytes* in a dextrose agar gel medium at 1000 ppm, and **8** and **14** were active against *Verticillium albo atrium* in the same agar medium at 1000 ppm.

In summary, introduction of an oxygen function into the 2 position of glycyrrhetic acid does not confer anti-DCA activity or antiviral activity to the compound. In addition, very little antiulcer activity is achieved, and only very low levels of antiinfective potency are attained.

Experimental Section

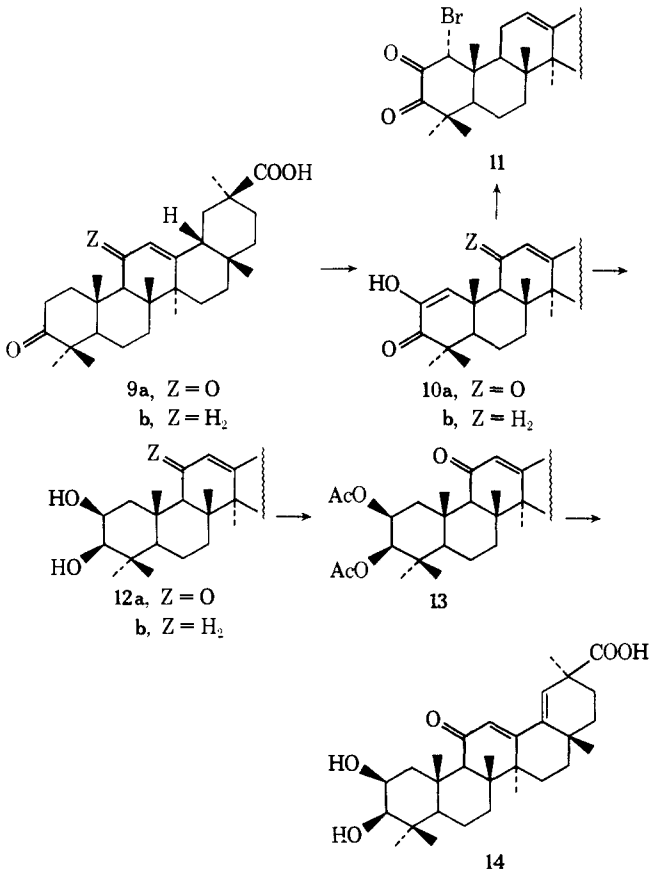
Melting points were taken on a Fisher-Johns hot-stage apparatus and are uncorrected. Melting points over 300° were taken on a Mel-Temp hot block apparatus and are also uncorrected. Nmr spectra were taken on a Varian A-60A, a Varian T-60, and Varian XL-100. All spectra are 60 MHz unless specified otherwise. Location of peaks (δ) is by parts per million away from TMS as an internal standard. Ir spectra were recorded in Chf unless specified otherwise, on a Beckman IR 12. Uv spectra were in MeOH on a Beckman DK-2A. Tlc runs were on 7.6-cm microscope slides covered with a 0.25-mm thickness of Woelm F silica, with a magne-

Scheme I



sium aluminum silicate binder. The solvents were PhH–EtOAc combinations. Visualization of spots was by uv light (254 nm) and by phosphomolybdic acid, 5% in EtOH (wt/vol), followed by heat. Column chromatography used Mallinckrodt SilicAR CC-4 and CC-7 silicic acid and Baker SiO_2. The weight ratio of absorbent to material was 100:1. Materials were applied as PhH solutions and, unless indicated otherwise, eluted with PhH containing increasing amounts of EtOAc. Materials were dried for analysis for 2 hr in an Abderhalden apparatus at 81° and 0.05 Torr unless specified otherwise. Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values. Decolorization was done with Darco G-60 charcoal. Evaporations were done under reduced pressure at or below 35°.

Scheme II



Methyl 2β-Acetoxy-3α-hydroxy-18β-olean-12-en-30-oate (2). Methyl 2β,3β-oxa-18β-olean-12-en-30-oate[2] (1.78 g, 3.70 mmol) was suspended in AcOH (20 ml) and warmed on a steam bath (90°) for 14 hr. The resulting solution was cooled and added to a suspension of prereduced PtO_2 (1.2 g) in AcOH (150 ml) and MeOH (7 ml). The mixture was pressurized to 39 psi with H_2 and agitated for 42 hr, the pressure being maintained between 14 and 39 psi. The reaction mixture was filtered, evaporated to a solid, dissolved in PhH, and applied to a CC-7 column. Elution with 98:2 PhH–EtOAc yielded **2** (976 mg, 50%). Continuation of this elution yielded the 3α-acetoxy-2β-hydroxy compound (*vide infra*). **2** was recrystallized from CH_2Cl_2–MeOH: mp 209–211°; nmr ($CDCl_3$) δ 4.98 (t, J = 7 Hz, 1 H) assigned to C_2H. *Anal.* ($C_{33}H_{52}O_5$) C, H.

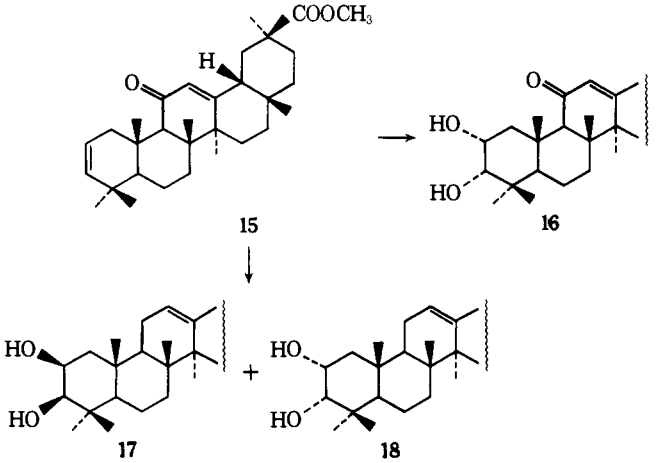
C_2H must be equatorial since J = 7 Hz, and therefore the C_2 acetoxy must be axial (β). The trans nature of the epoxide opening dictates an axial (α) configuration for the C_3 hydroxyl group.

Methyl 3α-Acetoxy-2β-hydroxy-18β-olean-12-en-30-oate (3). Continued elution of the CC-7 column described directly above with 95:5 PhH–EtOAc yielded **3** (701 mg, 35%) which was recrystallized from CH_2Cl_2–MeOH: mp 181–183°; nmr ($CDCl_3$) δ 3.96 (t, J = 7 Hz, 1 H), assigned to C_2H, 4.95 (d, J = 7 Hz), assigned to C_3H. *Anal.* ($C_{33}H_{52}O_5$) C, H.

The C_2H triplet (J = 7 Hz) shows that the proton must be equatorial, since for the axial case $J_{C(2)\text{-}H\text{-}C(1)\text{-}\beta\text{-}H} \cong$ 12–14 Hz. Therefore, C_2OH must be axial, or β. Since epoxide openings are trans, the C_3 OAc must therefore be α.

Methyl 2β,3α-Dihydroxy-18β-olean-12-en-30-oate (4). **3** (4.0 g, 7.58 mmol) was refluxed in 0.1 *N* KOH–MeOH (40 ml) for 20 min, followed by cooling to 5°. The resulting crystals were filtered, washed with cold MeOH and twice with H_2O, and crystal-

Scheme III



lized from EtOH: 1.8 g (48%); mp 265–267°. *Anal.* ($C_{31}H_{50}O_4$) C, H.

Methyl 2β,3α-Dihydroxy-11-oxo-18β-olean-12-en-30-oate (5). A mixture of methyl 2β-acetoxy-3α-hydroxy-11-oxo-18β-olean-12-en-30-oate (**1a**) and methyl 3α-acetoxy-2β-hydroxy-11-oxo-18β-olean-12-en-30-oate (**1b**) was synthesized as reported.[2] It was then dissolved (4.0 g, 7.38 mmol) in 0.1 *N* KOH–MeOH (50 ml), refluxed 5 min, and cooled. AcOH (1 ml) was added, the mixture was diluted to 125 ml with H_2O, and the resulting precipitate was filtered and washed twice with H_2O and crystallized (EtOH) giving 2.2 g (57%) of **5**, mp 277–279°. *Anal.* ($C_{31}H_{48}O_5$) C, H.

Proof of Structure of 1a,b. A mixture of **1a** and **1b** was separated by column chromatography on CC-4. PhH–EtOAc (8:2) eluted **1a** and then **1b**. Spectra for **1a**: nmr ($CDCl_3$) δ 4.97 (d, J = 8 Hz, 1 H) assigned to C_3H. Studies at 100 MHz ($CDCl_3$) show that ring A is a twisted boat, with $J_{C(2\alpha)\text{-}H\text{-}C(1\alpha)\text{-}H} = J_{C(2\alpha)\text{-}H\text{-}C(1\beta)\text{-}H}$ = 6.8 Hz; $J_{C(1\alpha)\text{-}H\text{-}C(1\beta)\text{-}H}$ = 14.5 Hz; and $J_{C(2\alpha)\text{-}H\text{-}C(3\beta)\text{-}H}$ = 8.1 Hz. Spectra for **1b**: nmr ($CDCl_3$) δ 4.91 (q, 1 H). Studies at 100 MHz ($CDCl_3$) show that ring A is again a twisted boat: $J_{C(2\alpha)\text{-}H\text{-}C(1\alpha)\text{-}H} = J_{C(2\alpha)\text{-}H\text{-}C(1\beta)\text{-}H}$ = 6.8 Hz; $J_{C(1\alpha)\text{-}H\text{-}C(1\beta)\text{-}H}$ = 14.8 Hz; $J_{C(2\alpha)\text{-}H\text{-}C(3\beta)\text{-}H}$ = 9.0 Hz. A decoupling irradiation of C_3H (329 Hz at 100 MHz) collapsed the C_2H quartet to a triplet (J = 6.8 Hz), verifying the assignments.

2β,3α-Dihydroxy-11-oxo-18β-olean-12-en-30-oic Acid (6). **5** (3.42 g, 6.82 mmol) was refluxed with $LiI \cdot H_2O$ (3.4 g, 22.4 mmol) in 2,4,6-collidine (80 ml) for 3 hr. In the beginning of reflux, the H_2O of hydration was distilled away. The cooled reaction mixture was partitioned between EtOAc–Et_2O–Chf and enough 4 *N* HCl to assure that the aqueous phase was acidic after the partition. The organic phase was washed three times with H_2O and then twice with saturated brine, decolorized, filtered, evaporated to a solid, and crystallized from CH_2Cl_2–MeOH and then from aqueous AcOH to give 1.16 g (35%), mp 311–314° dec. *Anal.* ($C_{30}H_{46}O_5$) C, H.

Methyl 2β,3α-Diacetoxy-11-oxooleana-12,18-dien-30-oate (7). A mixture of **1a** and **1b**[2] (7.85 g, 14.5 mmol) was acetylated with Ac_2O (20 ml) in Py (125 ml). The resulting isolated diacetate was dissolved[3] in glacial AcOH (100 ml) containing 48% HBr (14 drops) on a steam bath. A solution of Br_2 (0.8 ml) in glacial AcOH (15 ml) was added over 10 min. The reaction mixture was left on the steam bath another 10 min, diluted to 550 ml with cold H_2O, and filtered. The resulting solid was washed (H_2O) and partitioned between EtOAc and H_2O. The organic phase was washed with H_2O and then with saturated brine; it was then decolorized, filtered, evaporated to a solid, and crystallized (EtOH). The crystals (3.15 g) were chromatographed on CC-7 silicic acid. The product (3 g, 32%) was eluted with 9:1 PhH–EtOAc and crystallized (CH_2Cl_2–MeOH); mp 165–168°. *Anal.* ($C_{35}H_{50}O_7$) C, H. The mother liquor from the EtOH crystallization was evaporated to a solid (4.61 g) and subjected to the same chromatographic conditions to yield 2.8 g (29%) of pure material.

2β,3α-Dihydroxy-11-oxooleana-12,18-dien-30-oic Acid (8). **7** (1.45 g, 2.49 mmol) was refluxed in 0.5 *N* KOH–MeOH (35 ml) for 4.5 hr, cooled, diluted to 380 ml with H_2O, acidified (concentrated HCl), and filtered. The solid was washed (H_2O) and dried, yielding 919 mg (76%) of pure product, mp 208–215° dec. *Anal.* ($C_{30}H_{44}O_5$) C, H.

2-Hydroxy-3,11-dioxo-18β-oleana-1,12-dien-30-oic acid (10a) was prepared by the technique of Hanna and Ourisson.[4] **9a**[5] (3.0 g, 6.4 mmol), *t*-BuOH (46 ml), hexamethylphosphoric triamide (1.82 ml), and *t*-BuOK (3.78 g) were placed in a 500-ml Parr bottle and pressurized to 36 psi with O_2. After 10 min, the bottle was depressurized, H_2O (33 ml) was added, and the pH was adjusted to 3 (concentrated HCl). The mixture was concentrated to half its volume and then diluted to 200 ml (4 *N* HCl). The resulting solid was filtered, washed (H_2O), and dried, giving 3.0 g which was crystallized (Chf–EtOH), giving 1.0 g (32%) of pure **10a**, mp 308–312° dec. *Anal.* ($C_{30}H_{42}O_5$) C, H.

2-Hydroxy-3-oxo-18β-oleana-1,12-dien-30-oic acid (10b) was synthesized from **9b**[1] by the method given for **10a**. The product from the oxidation (91% mass recovery to give 4.2 g) was suspended in 100 ml of boiling EtOH–Chf (1:1), boiled down to 75 ml, kept at 5° overnight, and filtered to give 2.64 g (55%) of pure **10b**, mp 312–318° dec. *Anal.* ($C_{30}H_{44}O_4$) C, H.

1α-Bromo-2,3-dioxo-18β-olean-12-en-30-oic Acid (11). **10b** (1.1 g, 2.35 mmol) was dissolved in THF (50 ml) containing 48% HBr (3 drops). A solution of Br_2 in AcOH (0.5 N) was added dropwise with stirring until the yellow reaction mixture became orange. The reaction mixture was poured into aqueous $NaHSO_3$, and the resulting suspension was extracted twice with EtOAc. The combined organic fractions were dried ($MgSO_4$) and concentrated to a small volume. This was dissolved in hot EtOH, and the resulting solution was concentrated (N_2 stream) to 20 ml. The resulting canary yellow crystals were collected and washed (cold MeOH). A second crop was also collected. The crops were combined and crystallized (CH_2Cl_2–MeOH) to give 594 mg (46%) of pure **11**: mp 258–260° dec; nmr (C_5D_5N) δ 4.48 (s, 1 H) assigned to C_1H. This signal was not reduced by D_2O exchange. *Anal.* ($C_{30}H_{43}O_4Br$) C, H, Br.

In a similar bromination of a Δ^1-2-hydroxy-3-oxo-4,4-dimethyl-10-angular methyl steroid, Hanna and Ourisson[4] found that the product was the corresponding 1α-bromo-2,3-dioxo compound.

2β,3β-Dihydroxy-11-oxo-18β-olean-12-en-30-oic Acid (12a). **10a** (10 g, 20.7 mmol) was dissolved in THF (150 ml), 2-PrOH (80 ml), and H_2O (20 ml). The solution was cooled to 2° and $NaBH_4$ (7.2 g, 190 mmol) was added portionwise over 30 min, keeping the temperature at or below 12° (ice bath). The reaction proceeded another 40 min at 0–6° and was then quenched by pouring into 300 ml of H_2O (0°). The mixture was carefully acidified (concentrated HCl) and then extracted twice with Et_2O–EtOAc–CH_2Cl_2. The combined organic fractions were washed with dilute brine and then with saturated brine and then evaporated to a glass (12.1 g). The glass was crystallized (CH_2Cl_2–MeOH) to give 5.21 g (51%) of pure **12a**: mp 308–314° dec; nmr (C_5D_5N) δ 4.45 (m, 1 H) assigned to C_2H, 3.46 (d, J = 3.5 Hz, 1 H), assigned to C_3H. *Anal.* ($C_{30}H_{46}O_5$) C, H.

The C_2H signal is 14 Hz wide, and this implies an equatorial configuration for C_2H, and thus an axial (β) configuration for C_2OH. Studies with **1a**, **1b**, **2**, and **3** show that when the C_2H and C_3H signals are widely separated, as they are with **12a**, the two protons must be cis. Therefore, C_3OH must be cis to C_2OH, so the configuration is 2β,3β-dihydroxyl.

2β,3β-Dihydroxy-18β-olean-12-en-30-oic Acid (12b). **10b** (5.0 g, 10.6 mmol) was dissolved in THF–2-PrOH–H_2O (5:3:1 v/v/v), and $NaBH_4$ (3.0 g, 79 mmol) was added carefully. After 16 hr of reaction at room temperature, the mixture was diluted with H_2O (25 ml) and crushed ice (100 ml), carefully acidified (2 *N* H_2SO_4) to pH 2, diluted to 350 ml with H_2O, and extracted with EtOAc–Et_2O and then twice with EtOAc. The organic fractions were combined and washed twice with H_2O and once with saturated brine. The solid in the organic layer was filtered off (1.3 g) and suspended in boiling EtOAc (50 ml). The suspension was filtered and the solid retained. The filtrate of the organic layer from the extractions was evaporated to a solid (4.1 g) and suspended in boiling EtOAc (150 ml). The suspension was filtered and the solid was retained and added to the solid previously recovered from the boiling EtOAc filtration. The combined solids were washed with boiling Chf and then with boiling H_2O to give 2.5 g (49%) of pure **12b**: mp 301–310° dec; nmr (C_5D_5N) δ 3.45 (d, J = 4 Hz, 1 H), assigned to C_3H, 4.37 (m, 1 H), assigned to C_2H. The structure elucidation is as described for compound **12a**. *Anal.* ($C_{30}H_{48}O_4$) C, H.

2β,3β-Diacetoxy-11-oxo-18β-olean-12-en-30-oic Acid (13). **12a** (10.5 g, 21.5 mmol) was dissolved in Py (50 ml) and Ac_2O (25 ml) was added. After *ca.* 16 hr at room temperature, H_2O (25 ml) was added. After 30 min, the mixture was concentrated and then partitioned between Chf and 4 *N* HCl. The acidic aqueous layer was washed with Chf; the combined organic fractions were washed with H_2O and then with saturated brine. The organic solution was dried ($CaSO_4$), evaporated to a solid, and crystallized (CH_2Cl_2–MeOH) to give two crops which were combined and

crystallized again from the same solvents to give pure **13** (4.6 g, 37%), mp 311–320° dec. *Anal.* ($C_{34}H_{50}O_7$) C, H.

2β,3β-Dihydroxy-11-oxooleana-12,18-dien-30-oic Acid (14). **13** (11.2 g, 19.6 mmol) was treated as described for **7** through the decolorization step. After this, the decolorized solution was evaporated to a gum, which was dissolved in 0.5 *N* KOH–MeOH (100 ml) and refluxed 25 min. This reaction mixture was cooled, diluted to 1.1 l. (H_2O), acidified (4 *N* HCl), and extracted twice with EtOAc–Et_2O. The combined organic extracts were washed with H_2O, dilute brine, and saturated brine, decolorized, and evaporated to a solid which was crystallized (EtOH) to give three crops (2.93 g, 30%); mp 229–232°. *Anal.* ($C_{30}H_{44}O_5$) C, H.

Methyl 2α,3α-Dihydroxy-11-oxo-18β-olean-12-en-30-oate (16). **15**[2] (1 g, 2.15 mmol) was dissolved in 16 ml of a Py solution of OsO_4 (0.2 *M*) and heated on a steam bath for 70 min. The reaction mixture was allowed to stand overnight at room temperature, and then a solution of $NaHSO_3$ (0.1 g, 10 mmol) in H_2O (20 ml) was added.[10] The mixture was warmed on a steam bath for 10 min, cooled, and extracted twice with Et_2O. The combined organic phases were washed with 0.6 *N* HCl, H_2O, and then dilute K_2CO_3 solution, dried ($MgSO_4$), and evaporated to a gum. The gum was triturated with pentane to give 0.72 g of a solid which was crystallized (EtOH), giving 239 mg (22%) pure **16**: mp 238–239°; nmr (100 MHz, C_5D_5N) $J_{C(2)\text{-}H\text{-}C(3)\text{-}H}$ = 2.8 Hz; $J_{C(2)\text{-}H\text{-}C(1\beta)\text{-}H}$ = 4 Hz; $J_{C(2)\text{-}H\text{-}C(1\alpha)\text{-}H}$ = 12 Hz. *Anal.* ($C_{31}H_{48}O_5$) C, H. Assuming that the OsO_4 oxidation is a cis hydroxylation, and assuming that ring A is in a chair conformation, C_2H must be axial, because the large (12 Hz) *J* value cannot be derived from $J_{C(2)\text{-}H\text{-}C(3)\text{-}H}$ and thus must be from $J_{C(2)\text{-}H\text{-}C(1\alpha)\text{-}H}$. If C_2H is axial, C_2OH must be equatorial (α) and therefore C_3OH, being cis to C_2OH, must also be α, or axial. This calls for equatorial conformation for C_3H, and this is verified by the low $J_{C(2)\text{-}H\text{-}C(3)\text{-}H}$. See the preparation of compound **17** for decoupling experiments in a similar system.

Methyl 2β,3β-Dihydroxy-18β-olean-12-en-30-oate (17). **15**[2] (1.61 g) was treated as directly above again yielding a gum. This gum was hydrogenolyzed as in the preparation of **2**. The hydrogenolysis was repeated until tlc (7:3 PhH–EtOAc) showed very little unhydrogenolyzed material to be left. The material was then applied to a CC-7 column. Elution with 95:5 PhH–EtOAc gave **17** (634 mg, 38%), and a continuation of the column gave **18** (*vide infra*). **17** was crystallized from CH_2Cl_2–MeOH: mp 230–233° *Anal.* ($C_{31}H_{50}O_4$) C, H. Nmr (100 MHz, C_5D_5N): the C_2H is a quartet with a total width of 10 Hz. This *J* value is too small for an axial proton, so C_2H must be equatorial (α), meaning C_2OH must be β. C_3OH must thus also be β. To verify the assignment of peaks, the C_3H signal was irradiated, causing decoupling with the C_2H signal, which collapsed to a triplet (due to $C_{1\alpha H}$ and $C_{1\beta H}$ interactions with C_2H) as expected.

Methyl 2α,3α-Dihydroxy-18β-olean-12-en-30-oate (18). Continued elution of the column described directly above gave **18** with 9:1 PhH–EtOAc. **18** (310 mg, 18%) resulted and was crystallized (CH_2Cl_2–MeOH); mp 270–271° *Anal.* ($C_{31}H_{50}O_4$) C, H (structure by elimination).

Acknowledgments. I wish to thank Mr. Emanuel Zielinski for elemental analyses, Mr. Aristedes Damascus for determination of all spectra, Mr. William Selby and Mr. Michael Scaros for hydrogenolyses and high-pressure oxidations, Mr. Robert Nicholson for column chromatography, and Dr. Roy Bible for very helpful assistance in nmr interpretation. Special thanks are due to Dr. John Baran for many enlightening discussions and valuable suggestions.

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Synthesis of Tritium-Labeled Chlorambucil and Aniline Mustard of High Specific Activity

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Received April 16, 1973

Two procedures are described for the synthesis of 4-[4-bis(2-chloroethyl)amino-2-iodophenyl]butyric acid, a potential precursor to tritium-labeled chlorambucil. The longer synthetic route involves the stepwise elaboration of the two nitro groups in methyl 4-(2,4-dinitrophenyl)butyrate and defines the meta orientation of the iodine substituent with respect to the alkylating function. An alternative, single stage procedure involves the direct iodination of chlorambucil, using iodine in oleum (20% SO_3). This reagent also converted aniline mustard and melphalan into analogous iodo derivatives, *N,N*-bis(2-chloroethyl)-3-iodoaniline and 3-[4-bis(2-chloroethyl)amino-2-iodophenyl]-L-alanine, respectively. The reductive tritiation of the iodo derivatives of chlorambucil and aniline mustard yielded the appropriate ^3H-labeled drugs with specific activities respectively of 8.3 and 20.3 Ci/mmol. The percentages of the ^3H-labeled compound present in admixture with the unlabeled analog were independently estimated in each case by mass spectrometry.

Growing interest in the mechanism of the antineoplastic action of *N,N*-bis(2-chloroethyl)arylamines[1-3] has led to a need for radioactively labeled drugs of this class. ^3H-Labeled aniline mustard [*N,N*-bis(2-chloroethyl)aniline, **1**],[4] melphalan [3-[4-bis(2-chloroethyl)aminophenyl]-L-alanine, **2**],[5] and chlorambucil [4-[4-bis(2-chloroethyl)aminophenyl]butyric acid, **3**][1] have been synthesized by catalyzed tritium–halogen exchange of suitably iodinated derivatives. Only **1** has hitherto been obtained with high specific activity, and this fact has been attributed[4] to the meta relationship between the iodo and alkylating substituents in the precursor to ^3H-labeled **1**. An ortho relationship, such as existed in the precursors of **2** and **3**, was believed to account for most of the loss of radioactivity during the acidic conditions used for the isolation of ^3H-labeled **2**[5] and **3**.[1]

The objectives of the present investigation were the synthesis of ^3H-labeled chlorambucil of higher specific ac-