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NEW ENONE DERIVATIVES OF OLEANOLIC ACID AND URSOLIC ACID AS INHIBITORS OF NITRIC OXIDE PRODUCTION IN MOUSE MACROPHAGES

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Abstract: New derivatives of 3-oxoolean-1-en-28-oic acid and 3-oxours-1-en-28-oic acid were synthesized. Nine of them showed significant inhibitory activity against interferon-γ-induced nitric oxide production in mouse macrophages when assayed at the 1 μM level. 3,12-Dioxoolean-1,9-dien-28-oic acid (**3**) had the highest activity (IC_{50}, 0.9 μM). © 1997 Elsevier Science Ltd.

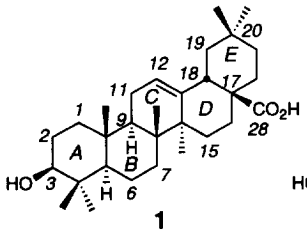
Introduction

Many oleanane and ursane triterpenoids are reported to have interesting biological, pharmacological, or medicinal activities similar to those of retinoids and steroids, such as anti-inflammatory activity, suppression of tumor promotion, suppression of immunoglobulin synthesis, protection of the liver against toxic injury, induction of collagen synthesis, and induction of differentiation in leukemia or teratocarcinoma cells.[1] However, there has never been a systematic study of structure–activity relationships in this set of molecules. Bioassay-directed systematic drug design and synthesis of derivatives of oleanolic acid (**1**) and ursolic acid (**2**), which are commercially available, are of great value in discovering new structures with significant biological activity.

The high output of nitric oxide (NO) produced by inducible nitric oxide synthase (*i*-NOS), which is expressed in activated macrophages, plays an important role in host defense. However, excessive production of NO also can destroy functional normal tissues during acute and chronic inflammation.[2] Thus, inhibitors of NO production in macrophages are potential anti-inflammatory drugs. For this purpose we synthesized oleanolic and ursolic acid derivatives and tested them as inhibitors of NO production. We have found a series of new derivatives of 3-oxoolean-1-en-28-oic acid and 3-oxours-1-en-28-oic acid to have significant inhibitory activity against interferon-γ (IFN-γ)-induced NO production in mouse macrophages.[3] In particular, 3,12-dioxoolean-1,9-dien-28-oic acid (**3**) had the highest activity (IC_{50}, 0.9 μM) in this group of compounds. In this communication, the synthesis, inhibitory activity, and structure–activity relationships are reported for these compounds.

Discovery of Lead Compounds

When we started this project, we had no information about a lead compound. Therefore, about sixty oleanolic and ursolic acid derivatives, e.g., 3-hydroxy-, 3-chloro-, 2-chloro-, C-ring cleaved, and 3-oxo-derivatives (including compounds **4**–**7**), were initially randomly synthesized. In the preliminary screen of these



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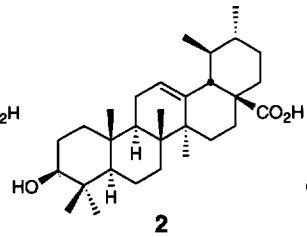

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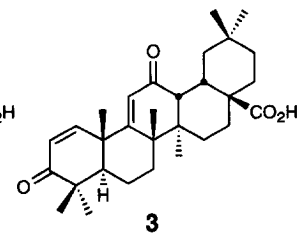

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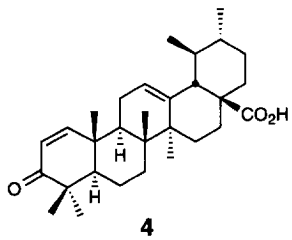

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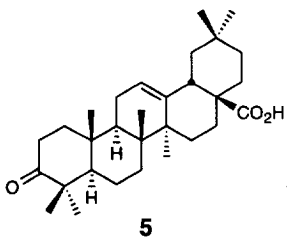

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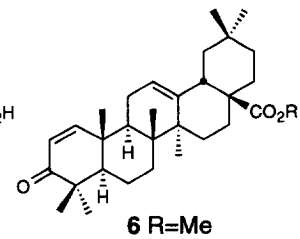

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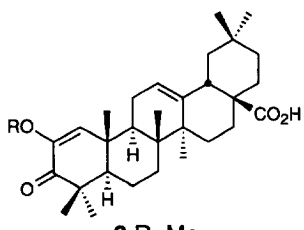

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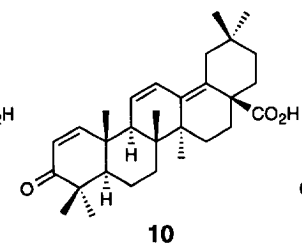

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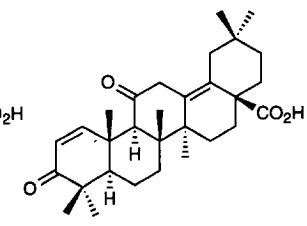

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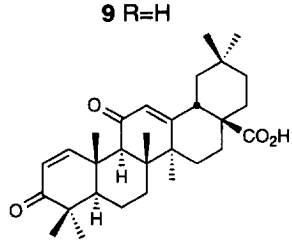

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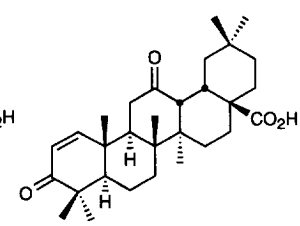

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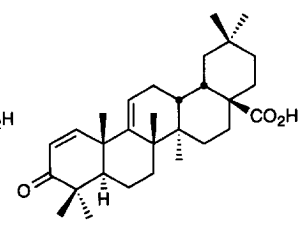

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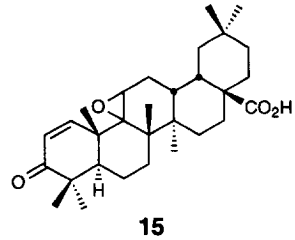

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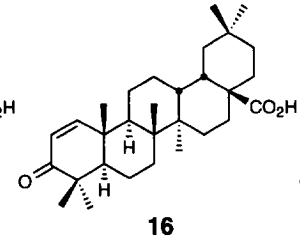

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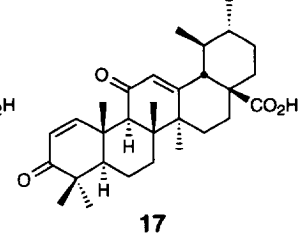

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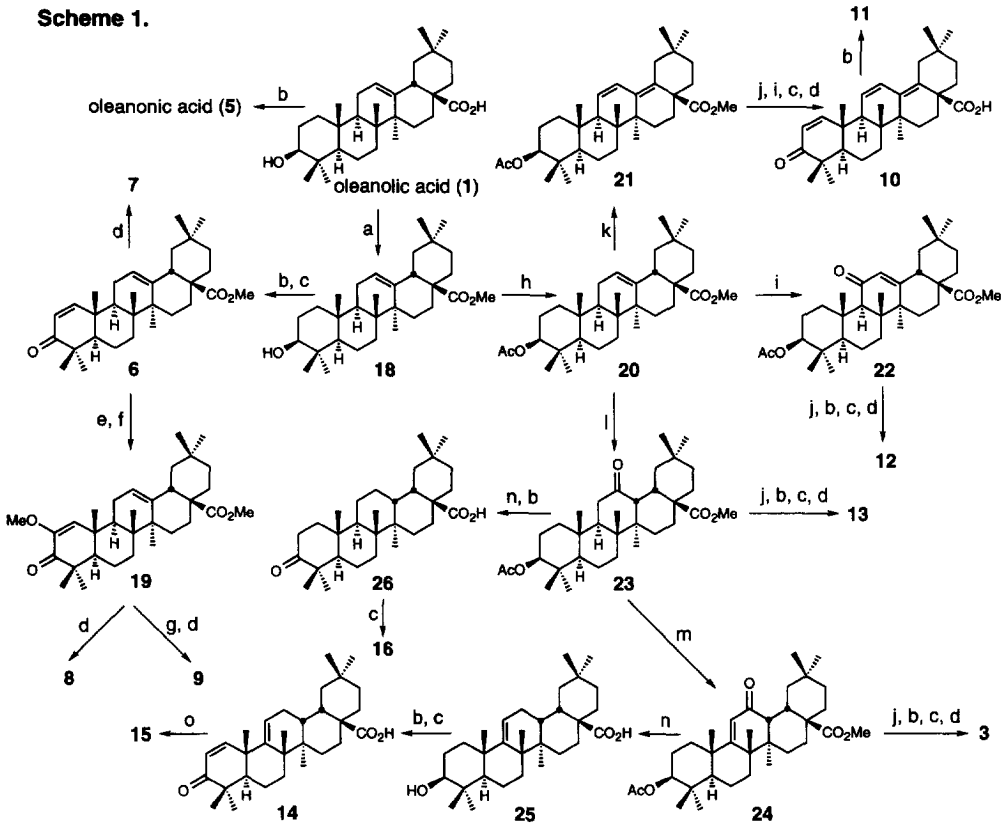
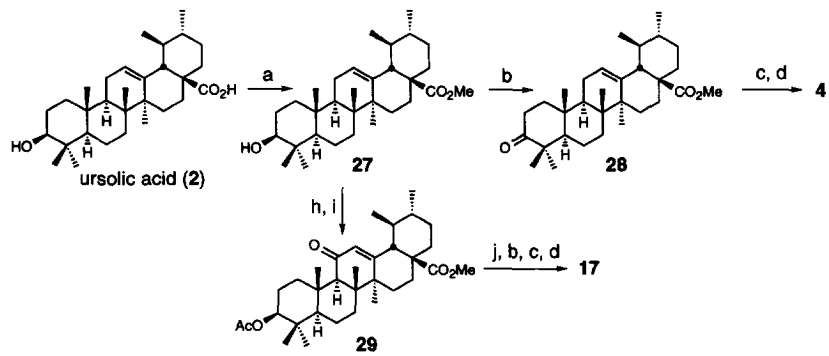
Scheme 1.



Scheme 2.



a: CH_2N_2/Et_2O/THF, b: Jones, c: PhSeCl/AcOEt; 30%H_2O_2/THF, d: LiI/DMF, e: 30%H_2O_2/NaOH/THF, f: MeONa, g: HCl/AcOH, h: Ac_2O/pyr., i: CrO_3/pyr./CH_2Cl_2, j: KOH/MeOH, k: SeO_2/AcOH, l: 30%H_2O_2/AcOH, m: Br_2/HBr/AcOH, n: NH_2NH_2/KOH/diethylene glycol, o: *m*-CPBA/CH_2Cl_2

derivatives for inhibition of IFN-γ-induced NO production in mouse macrophages, 3-oxoolean-1,12-dien-28-oic acid (**7**) was found to show significant activity (IC_{50}, 6.0 μM).

Design and Synthesis of New Derivatives

When **7** is compared with the other derivatives (e.g., **1**, **2**, and **4**–**6**), it has the following features: first, it is an oleanane; second, it has a 1-en-3-one structural unit in ring A; third, it has a carboxyl group at C-17. On the basis of these features of **7**, various derivatives with a 1-en-3-one structural unit in ring A and a carboxyl group at C-17 (**3** and **8**–**17**) were designed. The synthesis of these newly designed derivatives and compounds **4**–**7** are illustrated in Schemes 1 and 2.[4] Oleanonic acid (**5**)[5] was prepared in quantitative yield by Jones oxidation of **1**. Enone ester **6** was synthesized by Jones oxidation of methyl oleanolate (**18**)[6] (yield, 90%), followed by introduction of a double bond at C-1 with phenylselenenyl chloride in ethyl acetate and sequential addition of 30% hydrogen peroxide[7] ($PhSeCl\text{-}H_2O_2$) (yield, 70%). Enone **7** was synthesized in 88% yield by halogenolysis of **6** with lithium iodide (LiI) in dimethylformamide (DMF).[8] Enone **8** was synthesized in 35% yield by halogenolysis of ester **19** with LiI in DMF, which was prepared by epoxidation of **6** with alkaline hydrogen peroxide (yield, quantitative), followed by sodium methoxide (yield, quantitative).[9] Diosphenol **9** was synthesized by demethylation of the methyl enol ether at C-2 of **19** with hydrochloric acid in acetic acid (yield, 88%), followed by halogenolysis (yield, 18%). Diene **10** was synthesized by alkaline hydrolysis of acetate **21** (yield, quantitative), which was prepared from methyl acetyloleanolate (**20**)[6] according to a known method,[10] sequential Ratcliffe oxidation[11] (yield, 90%), introduction of a double bond at C-1 (yield, 66%), and halogenolysis (yield, 56%). Deconjugated enone **11** was prepared in 28% yield by Jones oxidation of **10**. Bis-enone **12** was synthesized by alkaline hydrolysis of acetate **22** (yield, quantitative), which was prepared from **20** according to our improvement on a known method,[12] sequential Jones oxidation (yield, 91%), introduction of a double bond at C-1 (yield, 97%), and halogenolysis (yield, 43%).[13] Enone **13** was synthesized in 46% yield from C-12 ketone **23**[14] according to the same synthetic route as for **12**. Bis-enone **3** was also synthesized in 26% yield from enone **24**[15] according to the same synthetic route as for **12**. Enone **14** was synthesized by Jones oxidation of acid **25**[16] (yield, 95%), followed by introduction of a double bond at C-1 (yield, 80%). Epoxide **15**[17] was prepared in 46% yield by epoxidation of **14** with *m*-chloroperbenzoic acid in methylene chloride. Enone **16** was prepared in 51% yield by introduction of a double bond at C-1 of acid **26**[18] with $PhSeCl\text{-}H_2O_2$. Enone **4** was prepared by introduction of a double bond at C-1 of ketone **28**[19] with $PhSeCl\text{-}H_2O_2$ (yield, 66%), followed by halogenolysis (yield, 88%). Bis-enone **17** was synthesized according to the same route as for **12** in 42% yield from enone **29**, which was prepared from **27** according to our improvement on a known method,[16,20]

Biological Results and Discussion

The inhibitory activities [IC_{50} (μM) value] of compounds **1**–**17** and hydrocortisone (a positive control) on IFN-γ-induced NO production in mouse macrophages are shown in the Table. Nine of the new derivatives of 3-oxoolean-1-en-28-oic acid and 3-oxours-1-en-28-oic acid showed significant activity at the 1 μM level. Six of them were superior to the lead compound **7**. Modification of the A and C ring affected activity strongly. In particular, bis-enone type compounds **3** and **12** showed high activity. Surprisingly, ursolic acid (**2**) stimulated NO production although ursolic acid derivatives **4** and **17** showed inhibitory activity. None of the synthesized derivatives were toxic to primary mouse macrophages at 40 μM.

These preliminary results revealed some interesting structure–activity relationships as follows:

(1) In the A ring, a 1-en-3-one structural unit without a substituent is important for significant activity. For example, 1-en-3-one **7** is much more active in comparison with diosphenol **9**, diosphenol methyl ether **8**, C-3 ketone **5**, and C-3 alcohol **1**.

(2) In the C ring: (a) a carbonyl group at C-11 and/or C-12 is important; (b) particularly, an insertion of a double bond at the α position of C-11 and/or C-12 ketone enhances the activity. Bis-enone **3** with 1-en-3-one and 9-en-12-one structural units showed the highest activity. Bis-enone **12**, C-11 ketone **11**, and C-12 ketone **13** also showed high activity, and were more active than **7**. Bis-enone **17** which has an ursane skeleton is also more active than **4**.

(3) At C-17, a carboxyl group (e.g., **7**) gives much more activity than a methoxycarbonyl group (e.g., **6**). Hydrophilic groups seem to be much better than hydrophobic groups.

(4) The oleanane skeleton is more active than the ursane skeleton. **7** and **12** are more active than **4** and **17**, respectively.

On the basis of these structure–activity relationships, further lead optimization is in progress. Studies on the mode of action of these derivatives also are in progress.

Table. IC_{50} (μM) [a] Values for Inhibition of IFN-γ-Induced NO Production in Mouse Macrophages [3]

Compound	IC_{50} (μM)	Compound	IC_{50} (μM)
hydrocortisone	0.015	**10**	9.7
3	0.9	**4**	17.6
12	1.8	**9**	26.5
11	2.6	**8**	30.0
13	3.3	**15**	35.5
17	5.1	**5**	37.1
14	5.2	**6**	40.0
7	6.0	oleanolic acid (**1**)	40.0
16	8.5	ursolic acid (**2**)	stimulation [b]

a: All IC_{50} (μM) values were determined over the range of 0.1–40 μM for each compound, except for hydrocortisone, using the computer calculation program Tablecurve® (all were fitted to a log–dose response curve.) Values are an average of two separate experiments.

b: Ursolic acid (**2**) is strongly toxic to primary mouse macrophages (toxic above 5–10 μM).

Acknowledgments

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3. Briefly, the procedure for this assay is as follows: Macrophages were harvested from female mice injected intraperitoneally four days previously with 4% thioglycollate. These cells were seeded in 96-well tissue culture plates and incubated with 4 ng/mL IFN-γ in the presence or absence of inhibitory test compounds. After 48 hours NO production (measured as nitrite by the Griess reaction) was determined. Full details of the assay are given in reference 21.
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