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# NOVEL A-RING CLEAVED ANALOGS OF OLEANOLIC AND URSOLIC ACIDS WHICH AFFECT GROWTH REGULATION IN NRP.152 PROSTATE CELLS

Heather J. Finlay,[a] Tadashi Honda,[a] Gordon W. Gribble,*[a] David Danielpour,[b] Nicole E. Benoit,[c]
Nanjoo Suh,[c] Charlotte Williams,[c] Michael B. Sporn.*[c]

[a]*Department of Chemistry, Dartmouth College, Hanover, NH 03755, U.S.A.*
[b]*National Cancer Institute, Bethesda, MD 20892, U.S.A.*
[c]*Department of Pharmacology and Toxicology, Dartmouth Medical School, Hanover, NH 03755, U.S.A.*

**Abstract:** Syntheses of eight novel A-ring cleaved oleanane and ursane analogs are described. These compounds were assessed for their ability to inhibit cell proliferation in NRP.152 prostate cells. Four A-ring cleaved derivatives showed significant activity; 5β-(1-methyl-2-ethyl)-10α-(3-aminopropyl)-des-A-urs-12-en-28-oic acid was the most active compound, ($IC_{50}$, 0.3 μM). © 1997 Elsevier Science Ltd.

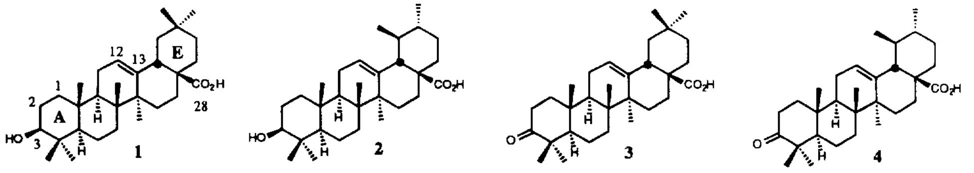
## Introduction:

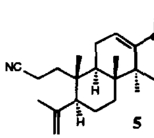
Triterpenoids form a large class of pentacyclic compounds derived biosynthetically from the cyclization of squalene.[1] Many of these naturally occurring compounds exhibit interesting biological and pharmacological properties.[2] For example, the closely related oleanolic acid (**1**) and ursolic acid (**2**) have been investigated for anti-inflammatory activity,[3] inhibition of tumorigenesis,[4] and modulation of collagen synthesis.[5] These commercially available[6] acids **1** and **2** are of particular interest for their ability to affect cell proliferation.[7]

Two epithelial cell lines, NRP.152[8,9] (nonmalignant) and NRP.154 (malignant), which demonstrate a sensitivity to retinoids and 1α,25-dihydroxyvitamin $D_3$, may be used for analysis of normal prostate growth and prostatic carcinogenesis. Compounds that inhibit the proliferation of nonmalignant cells mediated by the induction of TGF-β are, therefore, potential chemopreventive agents for prostate (and breast) cancer.[10] In this context, oleanolic acid, ursolic acid, and approximately 70 synthetic oleanane and ursane analogs were screened in the present study for their ability to affect growth regulation of NRP.152 prostate cells.

Four structurally modified triterpenoids, **9**–**12** showed significant growth inhibition at concentrations of <5 μM; 5β-(1-methyl-2-ethyl)-10α-(3-aminopropyl)-des-A-urs-12-en-28-oic acid, (**12**) was the most active compound in this series, $IC_{50}$, 0.3 μM. Syntheses of examples of this A-ring cleaved series (shown in Figure 1), inhibitory activity, and discovery of lead compounds **11** and **12** are described in the present letter.

**Figure 1**





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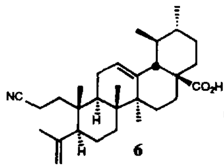

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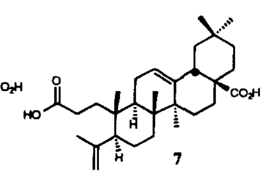

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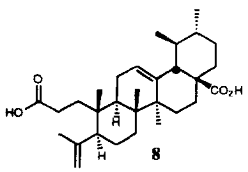

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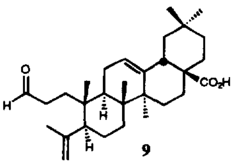

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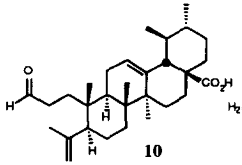

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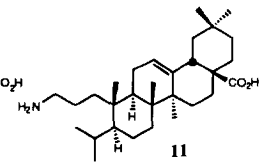

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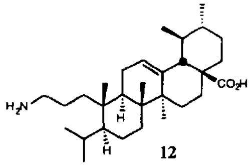

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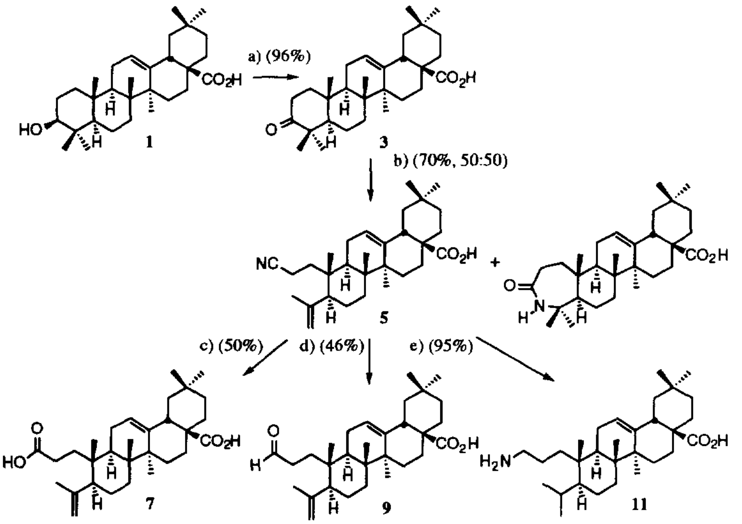
**Synthetic Modification of Oleanolic and Ursolic Acids:**

Jones oxidation[11] of oleanolic acid furnished intermediate ketone **3** (96%), which was cleaved using the Schmidt reaction conditions described[12] (Scheme 1). These conditions proved superior to those reported using hydroxylamine-*O*-sulfonic acid[13] for this substrate. A-ring cleaved nitrile **5** was obtained in 35% yield after flash column chromatographic separation from the lactam by-product. The nitrile was subsequently converted to the corresponding carboxylic acid **7** by a tetrafluorophthalic acid melt[14] (50%), to the aldehyde **9** by DIBAL reduction[15] (2.0 equiv in THF, 46%), and to the related primary amine **11** by hydrogenation over catalytic 10% palladium on activated charcoal[16] (95%). The C12-13 was not reduced under the hydrogenation conditions due to the sterically hindered nature of this double bond.[17] An analogous sequence of transformations lead to the A-ring cleaved ursanes from ursolic acid. Thus, oxidation and A-ring cleavage of **2** generated nitrile **6** (71%, 40:60 ratio of nitrile:lactam), and subsequent nitrile hydrolysis[18] (56%), DIBAL reduction[15] (81%), and hydrogenation[16] (68%) furnished **8**, **10**, and **12** respectively.

**Scheme 1**



(a) Jones Oxidation; (b) $NaN_3/AcOH/H_2SO_4$; (c) Tetrafluorophthalic acid melt/160 °C; (d) 2.0 equiv DIBAL/-30 °C/THF; e) $H_2$/10%Pd/C/EtOH/10%HCl.

**Table 1**: Inhibitory Activity of Triterpenoids on NRP.152 Prostate Cell Proliferation.

| Compound[19] | $IC_{50}$[a] (μM) | Compound[19] | $IC_{50}$[a] (μM) |
|---|---|---|---|
| TGF-β | 0.000014 | **1** | >5.0 |
| **12** | 0.3 | **2** | >5.0 |
| **11** | 0.7 | **3** | >5.0 |
| **9** | 1.5 | **4** | >5.0 |
| **10** | 2.4 | **7** | >5.0 |
| **5** | 3.8 | **8** | >5.0 |
| **6** | >5.0 | | |

(a) $IC_{50}$ values for the range 0.1 – 5.0 μM for all compounds except TGF-β calculated using the program Tablecurve®. Values are fitted to a log-dose response curve.

## Biological Results and Discussion:

Oleanolic acid, ursolic acid, synthetic derivatives **3** – **12** and approximately 70 other structurally modified oleananes and ursanes were screened in vitro for their ability to inhibit proliferation of nonmalignant, non-tumorigenic prostate cells.[8,9] Aldehydes **9** and **10** were active at a concentration of 2 μM ($IC_{50}$, 1.5 and 2.4 μM respectively), but amines 5β-(1-methyl-2-ethyl)-10α-(3-aminopropyl)-des-A-olean-12-en-28-oic acid (**11**) and the corresponding ursane **12** were the most active compounds of those tested ($IC_{50}$, 0.7 and 0.3 μM, respectively). Table 1 lists the $IC_{50}$ values (μM) for compounds **1** – **12** and the positive control TGF-β.

From these results there are three preliminary structure–activity relationships:

(i) The corresponding ursane and oleanane derivatives have similar activities (**1** and **2**, **3** and **4**, **5** and **6**, **7** and **8**, **9** and **10**, **11** and **12**).

(ii) The A-ring cleaved compounds are more active than oleanolic acid and ursolic acid and the 3-oxo derivatives (**5**–**12** are more active than **1**–**4**).

(iii) Conversion of nitriles **5** and **6** to the corresponding aldehydes **9**, **10** and reduction of the nitriles to the amines **11** and **12** results in significantly increased activity.

Clearly, modification of ring A with subsequent further reduction of the intermediate nitrile is essential for activity. It is not yet clear whether reduction of both the 3-nitrile and the 4-alkene functionality are required. Amines **11** and **12** will be adopted as lead compounds. Further modification of these derivatives and synthesis of A-ring cleaved analogs are currently in progress.

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