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THE CHEMISTRY OF TOXIC PRINCIPLES FROM *MAYTENUS NEMEROSA*

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Key Word Index—*Maytenus nemerosa*; Celastraceae; cytotoxic principles; antineoplastic agents; pentacyclic triterpenes.

Abstract—*Maytenus nemerosa* has been fractionated systematically by following the toxicity of extracts, partitioned solutions and column chromatographic fractions against cultured KB cells. Several compounds were isolated in this fashion, including 3-oxo-20(29)-lupen-30-al, β-amyrin, 29-hydroxyfriedelan-3-one, 30-hydroxy-20(29)-lupen-3-one, 30-hydroxyfriedelan-3-one and lup-20(29)-ene-3β,30-diol, as well as tingenone, 20-hydroxytingenone and galactitol. 3-Oxo-20(29)-lupen-30-al was shown to be cytotoxic for the first time; the structural basis of this cytotoxicity was investigated, in part, by bioassay of four products obtained by chemical transformation of single, isolated principles.

INTRODUCTION

Maytenus species have been studied extensively and shown to produce a variety of metabolites, including those of the ansamacrolide class [1, 2]. Further investigation of *Maytenus nemerosa* has now yielded a group of lupane triterpenes, some of which possess moderate cytotoxicity towards KB cells *in vitro*. Comparison of the toxicities of these metabolites, and of several analogs obtained from them by synthetic transformations, has permitted correlation of cytotoxicity with certain of the structural features in these molecules.

RESULTS AND DISCUSSION

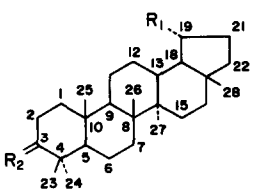
Isolation and identification of principles

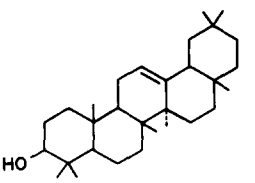
The hexane extract of the dried wood and stems of *Maytenus nemerosa* demonstrated significant inhibitory activity *in vitro* against KB cells. This extract was partitioned between 20% aqueous methanol and petrol; each of these fractions was cytotoxic toward KB cells and both were studied further.

The residue from the petrol layer was fractionated by chromatography on silica gel; the least polar metabolite (1) possessed KB cytotoxicity and gave a positive Liebermann–Burchard test for triterpenes. This assignment was consistent with the data obtained by high resolution mass spectrometry ($C_{30}H_{46}O_2$; *m/z* calc. 438.3486, found 438.350). The UV spectrum of 1 had λ_{max} 227 nm (ε1970), indicating the presence of an α,β-unsaturated carbonyl group and the IR had absorptions at 1695 and 1686 cm^{-1} (ketone in a six-membered ring and an α,β-unsaturated aldehyde, respectively). An olefinic methylene group was suggested by absorptions at 1640, 925 and 900 cm^{-1} [3].

The 360 MHz ^1H NMR spectrum of 1 contained six methyl singlets (at δ 0.83, 0.92, 0.94, 1.02, 1.05 and 1.07), a singlet at δ 9.52 (vinylic aldehyde) and two singlets (δ 5.90 and 6.29) that were assigned as the β-H's of an α-substituted enone system. Catalytic hydrogenation of 1 (Pd–C, methanol, 1 atm H_2) resulted in the incorporation of one equivalent of H_2 and the formation of a new compound (7) whose ^1H NMR spectrum lacked the olefinic protons but contained a methyl signal at δ 1.13 (*d*, *J* = 6 Hz). This signal was coupled to the same proton (C-20 H) that was coupled both to the aldehydic proton (δ 9.88, *d*, *J* = 3 Hz) and to C-19 H. These data were sufficient to establish the presence of the conjugated aldehyde–olefinic methylene system at C-19.

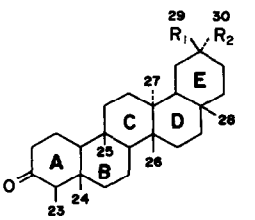
Comparison of the chemical shifts of the tertiary methyl groups in 1 with those of lup-20(29)-ene-2α,3α diol [4] and other lupene derivatives [5] suggested their occurrence at C-23 (δ 1.05), C-24 (δ 1.02), C-25 (δ 0.92), C-26





2

	R_1	R_2
1	OHC	O
4	HO	O
6	HO	α-H, β-OH
7	OHC	O
8		O
9	HO	O
10	OHC	α-H, β-OH



3 R_1=CH_2OH, R_2=Me
5 R_1=Me, R_2=CH_2OH

(δ 1.07), C-27 (δ 0.94) and C-28 (δ 0.83) of a lupane-type skeleton [6]. The mass spectrum and ^{13}C NMR spectrum of **1** were found to be very similar to those of lupenone (**10**) [7, 8]. Confirmation of the assigned structure was obtained by oxidation of **6** to **1** (CrO_3–pyridine). After completion of the structure elucidation of **1**, the same compound was isolated from *Gymnosporia emarginata* (Celastraceae) [9], albeit without any report of biological activity.

The second of the six crystalline compounds that eluted from the silica gel column described above was β-amyrin (**2**), a known compound [10]. This was followed by another solid compound that deposited colorless needles (chloroform–petrol), mp 275–278°. The molecular formula of this triterpene (**3**) was established as $C_{30}H_{50}O_2$ (*m/z* calc. 442.3811, found 442.381). This compound exhibited only end absorption in the UV; an absorption at 3550 (*br*) cm^{-1} indicated the presence of a hydroxyl group and another at 1710 cm^{-1} was assigned to a ketone in a six-membered ring. The electron-impact mass spectrum of **3** had fragment ions at *m/z* 342, 302 and 273, suggesting that the compound contained the friedelane skeleton with the carbonyl moiety, but not the hydroxyl group, in rings A, B or C [11]. The ^1H NMR spectrum of **3** indicated the presence of six tertiary methyl groups and one secondary methyl group; a resonance at δ 3.39 (*m*, 2) was assigned to $-CH_2OH$, suggesting that the hydroxyl group was attached to C-28, C-29, or C-30. Comparison of the physical and spectral data obtained for compound **3** with the data recorded for 29-hydroxyfriedelan-3-one [12, 13] indicated that the two were identical.

The fourth compound to elute from the silica gel column crystallized from ether–petrol as colorless prisms, mp 183–184°. The molecular formula of compound **4** was shown to be $C_{30}H_{48}O_2$ (*m/z* calc. 440.3654, found 440.365) and the IR spectrum contained absorptions at 3550, 1690, 1640 and 880 cm^{-1}. In addition to resonances assigned as six tertiary methyl groups, the ^1H NMR spectrum of **4** contained signals corresponding to one hydroxymethyl group (δ 4.12, *s*), two olefinic protons (δ 4.91 and δ 4.94) and a hydroxyl group (δ 1.33). That the isolated compound had structure **4** was established by comparison of the spectral data with those of authentic 30-hydroxy-20(29)-lupen-3-one (**4**), which has been isolated from *Gymnosporia emarginata* [9] and *Flourensia heterolepsis* [14].

Hydrogenation of a methanolic solution of **4** (5% Pd–C, H_2) provided two new compounds. One of these was shown to be lupenone (**8**) by comparison of infrared, ^1H NMR and mass spectral data with those published for authentic lupenone [7]. The second hydrogenation product, isolated in low yield, was found to have a molecular ion at *m/z* 442, suggesting the molecular formula $C_{30}H_{50}O_2$. On the basis of its IR and ^1H NMR spectra, this product was formulated as **9**. Treatment of **9** with $POCl_3$ in pyridine effected conversion to lupenone (**8**), suggesting that the conversion of **4** to **8** may involve the intermediacy of **9**.

The fifth compound (**5**) eluted from the silica gel column (colorless needles, mp 271–273°) was shown to have the molecular formula $C_{30}H_{50}O_2$ (*m/z* calc. 442.3811, found 442.381). The mass spectral fragmentation pattern suggested that this compound had the friedelane skeleton. Compound **5** was found to have a ^1H NMR spectrum similar to that of **3**, but the $-CH_2OH$ signal resonated at δ 3.26, and the corresponding signal for the *O*-acetate at δ 3.75. This was sufficient to establish the structure of **5** as 30-hydroxyfriedelan-3-one, a species isolated previously from *Salacia fraticosa* [15] and *Catha cassinoides* [13].

The most polar compound (**6**) eluted from the silica gel column was obtained as colorless needles from chloroform–ethanol. It had a molecular formula of $C_{30}H_{50}O_2$ (*m/z* calc. 442.3811, found 442.381). The IR spectrum indicated the presence of hydroxyl (3300 cm^{-1}) and olefinic methylene (1640 and 890 cm^{-1}) groups, but no carbonyl moiety. In common with **4**, the ^1H NMR spectrum of compound **6** reflected the presence of six tertiary methyl groups, a hydroxymethyl (δ 4.05 and δ 4.14) group and two olefinic protons (δ 4.89). The presence of two hydroxyl groups was inferred from the smooth conversion of **6** to the respective di-*O*-acetate, isolated as colorless prisms from methanol–ether, mp 143–144°. These data were sufficient to establish that **6** was identical with lup-20(29)-ene-3β,30-diol, a compound isolated previously [4, 9, 16]. Oxidation of **6** (CrO_3–pyridine, 0°, 18 hr) gave both **1**, identified by comparison with an authentic sample, and 3β-hydroxy-20(29)-lupen-30-al (**10**), whose spectral properties were identical with those of the same compound isolated from *Quercus championi* [3].

The aqueous methanol layer resulting from partition of the hexane extract was also found to be cytotoxic toward KB cells. Two major constituents were isolated; these were identified as tingenone and 20-hydroxytingenone by comparison of spectral data with those reported for these compounds and by comparison of isolated tingenone with an authentic sample [17]. Tingenone and 20-hydroxytingenone have been isolated previously from *Euonymus tingens* [18] and *Maytenus rigida* [19]. The plant material remaining after successive extractions with hexane and ether was extracted with methanol. This methanol extract was partitioned between chloroform and water and an insoluble component was crystallized from water as colorless prisms, mp 185–187°. This compound could be converted to a crystalline hexaacetate, mp 167–168°, and was shown to be identical with galactitol by comparison of physical data with those of the authentic compound [20].

Biological assays

Compounds **1**–**10** were assayed for cytotoxicity toward cultured KB cells. Compound **1** was found to be cytotoxic at a concentration of 10 μg/ml, although none of the structurally related compounds tested (i.e. **4**, **6**–**10**) exhibited significant inhibition of KB cell growth at the same concentration. Comparison of the structures of these compounds leads to the conclusion that expression cytotoxicity by compound **1** requires the presence of the carbonyl moiety at C-3 (cf. compound **10**) and of the α,β-unsaturated aldehyde moiety at C-19 (cf. compounds **4**, **7**–**9**). This finding is consistent with observations in other structural series of compounds regarding the contribution of two electrophilic groups in a single molecule toward the expression of antineoplastic activity [21, 22]. Also active as cytotoxic agents were tingenone and hydroxytingenone, consistent with previous observations for these and structurally related species [23, 24].

EXPERIMENTAL

^{1}H NMR spectra were taken in $CDCl_3$ on a Varian EM-390 spectrometer or a NT 360 MHz spectrometer. ^{13}C NMR spectra were recorded on a JEOL-100 NMR spectrometer operating at 25.15 MHz in the Fourier transform mode.

Isolation procedures. Dried wood and stems of *Maytenus nemerosa* (412 g) were cut in pieces and soaked with four portions of hexane over a period of 12 days. The hexane extract (3.17 g) was partitioned between 20% aq. MeOH (50 ml) and petrol (50 ml); the MeOH soln was back-extracted with two 50 ml portions of petrol. Concn of the petrol layer gave 2.98 g of extract; 0.13 g was isolated from the methanol layer. The extract from the petrol layer was dispersed onto 5 g of silica gel and placed at the top of a 60 g (2.4 × 42 cm) silica gel column. The column was washed with mixtures of petrol and Et_2O, and column fractions were combined according to analytical TLC results.

Hydrogenation of 3-oxo-20(29)-lupen-30-al. A soln containing 60 mg of compound **1** in 10 ml MeOH was hydrogenated over 1% Pd–C (1 atm H_2) for 15 min. Chromatography of the product (silica gel column; 9 g; 1.3 × 21 cm), elution with mixtures of petrol and Et_2O, provided 3-oxo-lupan-30-al (**7**), which crystallized as colorless plates from EtOH–Et_2O in low yield, mp 184–186°; ^{1}H NMR ($CDCl_3$): δ 0.79 (*s*, 3), 0.96 (*s*, 3) 0.97 (*s*, 3), 1.06 (*s*, 3), 1.12 (*s*, 6), 1.13 (*d*, 3, J = 6 Hz) and 9.88 (*s*, 1); MS *m/z*: 440 $[M]^+$ and 425.

Hydrogenation of 30-hydroxy-20(29)-lupen-3-one. A soln of 41 mg (94 μmol) of compound **4** in 10 ml MeOH was hydrogenated over 5 mg of 5% Pd–C (1 atm H_2) for 2 hr. The reaction mixture afforded two products separable by chromatography on silica gel. The less polar product crystallized (EtOH–Et_2O) as colorless needles, yield 18 mg (44%), mp 165–166°; $[\alpha]_D^{24}$ + 51.1° (*c* 0.54, $CHCl_3$); ν_{max}^{KBr} cm^{-1}: 1710, 1640 and 880; ^{1}H NMR ($CDCl_3$) δ 0.80 (*s*, 3), 0.93 (*s*, 3), 0.95 (*s*, 3), 1.02 (*s*, 3), 1.05 (*s*, 6), 1.67 (*s*, 3), 2.42 (*m*, 2) and 4.60 (*d*, 2, J = 15 Hz); MS *m/z*: 424 $[M]^+$, 409, 381, 218, 205 and 189. The spectral data was consistent with the assignment of this compound as lupenone (**8**) [6, 12]. The more polar compound was obtained as colorless needles (EtOH–Et_2O), yield 0.9 mg, mp 285–287°; ν_{max}^{KBr} cm^{-1}: 3540, 2930, 2850, 1710, 1465, 1450, 1390, 1200, 1050, and 1030; ^{1}H NMR ($CDCl_3$): δ 0.73 (*s*, 3), 0.88 (*d*, 3, J = 5 Hz), 0.89 (*s*, 3), 1.00 (*s*, 3), 1.01 (*s*, 3), 1.07 (*s*, 3), 1.15 (*s*, 3), 2.28 (*m*, 2) and 3.40 (*m*, 2); MS *m/z* 442, 427 and 424.

Oxidation of lup-20(29)-ene-3β,30-diol (**6**). Compound **6** (9.5 mg, 20 μmol) was dissolved in 1 ml of pyridine and treated with 10 mg (10 μmol) of CrO_3 at 0° for 18 hr. The reaction mixture was then treated with ice water and the precipitated solid was filtered. The dried solid was fractionated by prep. TLC on silica gel, development with 1.5% MeOH in $CHCl_3$. Two products were isolated by this procedure. The first of these (0.7 mg) was shown to be 3-oxo-20(29)-lupen-30-al (**1**) by direct comparison with an authentic sample. The second product of the oxidation reaction (**10**, 2.3 mg) formed colorless needles from EtOH, mp 228–229°; $[\alpha]_D^{24}$ + 2.3° (*c* 0.086, $CHCl_3$); λ_{max} (MeOH) 228 nm (ε7800); ν_{max}^{KBr} cm^{-1}: 3310, 2960, 2890, 1690, 1630, 1475, 1465, 1400, 1390, 1375, 1200, 1110, 1040, 1030, 1010, 980, 950 and 870; ^{1}H NMR ($CDCl_3$) δ 3.20 (*dd*, 1), 5.86 (*s*, 1), 6.23 (*s*, 1), and 9.51 (*s*, 1); MS *m/z*: 440, 425, 407, 207, 203, 189 and 187.

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