Bioorganic & Medicinal Chemistry Letters 23 (2013) 3542–3546

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

A bioassay-driven discovery of an unexpected selenophene and its cytotoxicity

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ARTICLE INFO

Article history:
Received 13 March 2013
Revised 11 April 2013
Accepted 13 April 2013
Available online 23 April 2013

Keywords:
Glycyrrhetinate
SeO_2 oxidation
Selenophene
Cytotoxic
Apoptosis

ABSTRACT

During the reaction of methyl 3β-acetoxy-glycyrrhetinate (**1**) with SeO_2 significant amounts of a cytotoxic hitherto unprecedented triterpenoic selenophene **3** are formed. This compound stops cell proliferation and acts by apoptosis.

Almost seven decades ago Ruzicka and co-workers[1] described the 'oxidation' of methyl 3β-acetoxy-glycyrrhetinate (**1**, Fig. 1) with SeO_2 leading to an unusual product **2** of unknown structure. As an alternative[1,2] to SeO_2 the use of CrO_3 was suggested, but the structure of **2** remained unknown at that time. Several years later, McKean and Spring[3] identified this compound as an α,β-unsaturated lactone, the structure of which was deduced from additional experiments[4,5] including derivatization, combustion analysis and stereochemical considerations. The unusual structure of an anellated enol-lactone is not limited to glycyrrhetinic acid derivatives but was also found for other triterpenoids[6,7] of the oleanane type. Finally, in 2010 the structure of **2** was secured[8] by NMR data and a single crystal X-ray analysis. Hence, the 'oxidation' of **1** introduces two more oxygen atoms, it eliminates four hydrogens, it re-arranges one ring, and it generates an additional unsaturated γ-lactone ring.[8] Although compound **2** has been known for decades, no biological data have been published so far.

During our continuing investigation of antitumor active derivatives of glycyrrhetinic acid derivatives, we became interested in the synthesis and biological evaluation of this type of compound. Thus, reaction of **1** with SeO_2 as described (AcOH, SeO_2, 120 °C, 24 h)[1,8] gave a mixture of many compounds as indicated by TLC. In a bioassay-driven LC fractionation several of these products (often as a mixture of compounds as indicated by ESI-MS) were isolated by preparative LC, and they were tested for their cytotoxic activity using a photometric SRB assay employing human tumor cell lines and mouse fibroblasts NiH 3T3. As a result, two fractions were identified containing cytotoxic compounds: The first bio-active fraction contained lactone **2**, the second fraction consisted of an unknown side-product **3**[9] that was formed in significant amounts (up to 15% isolated yield).

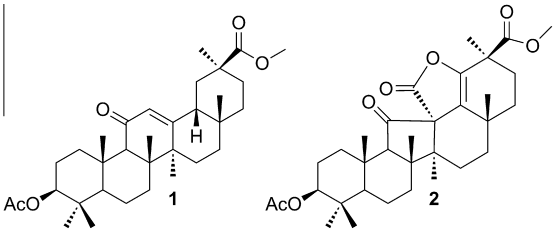

Figure 1. Structure of **1** and 'oxidation product' **2**.

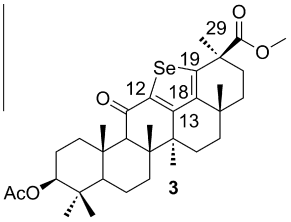

Figure 2. Structure of the unexpected selenophene **3**.

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http://dx.doi.org/10.1016/j.bmcl.2013.04.036

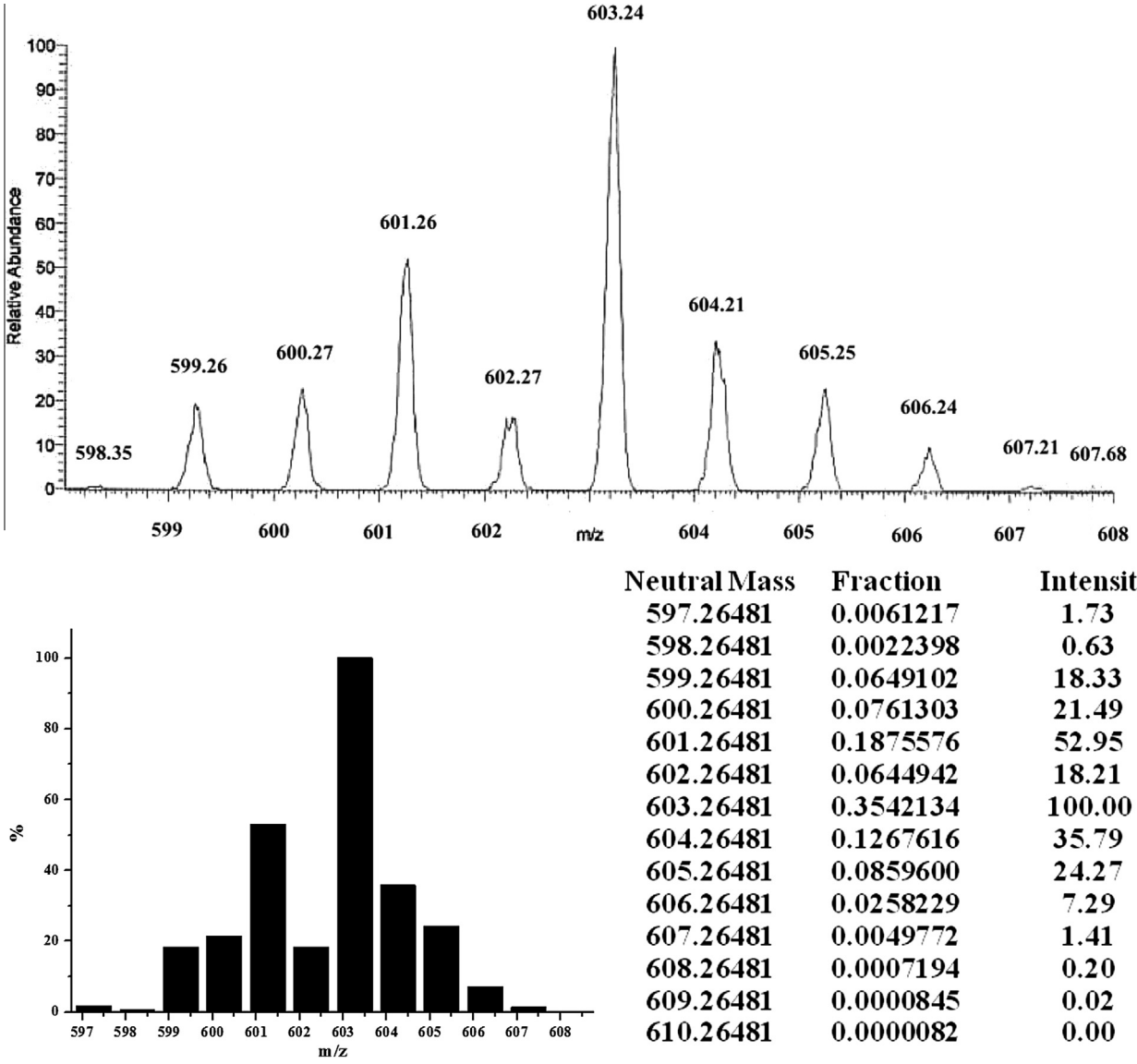

Figure 3. Experimental and calculated isotopic distribution for the quasimolecular ion $[M+H]^+$ of compound **3**.

Close inspection of the ^1H and ^{13}C NMR data of **3** revealed the absence of protons at C-18 and C-19 as well as at C-12 and C-13. The protons $H_3C(29)$ are shifted to lower fields (δ = 1.48 ppm). An additional ^{77}Se NMR spectrum revealed the presence of a signal at δ = 592.27 ppm being typical[10] for a selenophene. In the IR spectrum typical C=Se stretching vibrations[11] were found at ν = 684 and 1469 cm^{-1}.

An ESI-MS experiment showed the presence of a quasimolecular ion $[M+H]^+$ m/z = 603.3 with a rather complex pattern (Fig. 2) corresponding to a molecular formula of $C_{33}H_{46}O_5Se$. The calculated isotope distribution pattern for this molecule is also depicted in Figure 3 and matches the result from the experiment.

^{75}Se labeled derivatives have been used as pancreatic imaging agents[12] using γ-ray scintigraphy, and several selenium compounds[13] were discussed in the chemoprevention of colon-cancer representing one of the leading causes[14] for cancer-associated death in the Western world.[15–17] Thus, selenium compounds have been suggested[15–17] as potent novel cytotoxic agents leading to an apoptotic response[18] either by a caspase-dependent process[19] or by their interaction[15] with glutaredoxin proteins.

Recently, the cytotoxicity of selenophenes has been investigated in more detail. Thus, for some benzo[*b*]selenophene derivatives cytotoxic activity on human fibrosarcoma cell has been established,[20] and for compound D-501036 (a bis-(2-selenienyl)-*N*-pyrrole derivative) Chang and co-workers[21] showed, that this compound induces cellular apoptosis through the p53-associated mitochondrial pathway. Also, several selenophenyl-substituted quinolines[22] as well as selenocarbamates[23] exhibited significant cytotoxic activity. Hence, we became interested in the cytotoxic activity of **3** as compared to its parent compound **1** and lactone **2**.

Table 1
Cytotoxicity (IC_{50} values in μmol from SRB assays after 96 h of treatment; averaged results from three independent experiments and using the two-parametric Hill-slope equation using Graphpad Prism 5.04 and JMP7 software for calculations) of **1**–**3** against a panel of selected human tumor cell lines and a nonmalignant mouse fibroblast cell line (NiH 3T3)

Cell line	1	2	3
518A2	26.1 ± 1.6	14.7 ± 0.6	36.7 ± 0.5
A2780	40.5 ± 3.7	19.7 ± 3.3	33.7 ± 0.6
MCF7	51.6 ± 2.6	33.5 ± 1.3	45.6 ± 3.3
8505C	116.4 ± 10.7	73.2 ± 5.5	42.2 ± 2.8
HT29	77.4 ± 7.7	58.7 ± 6.6	67. 2 ± 7.8
A549	42.6 ± 7.3	15.0 ± 0.9	46.9 ± 1.8
NiH 3T3	101.4 ± 7.2	38.1 ± 4.3	46. 1 ± 4.4