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Separation and identification of some common isomeric plant triterpenoids by thin-layer chromatography and high-performance liquid chromatography

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ABSTRACT

Chromatographic separation of 10 triterpenoids (α-amyrin, β-amyrin, δ-amyrin, lupeol, lupenon, lupeol acetate, cycloartenol, cycloartenol acetate, ursolic acid, oleanolic acid) and 2 sterols (stigmasterol and β-sitosterol) was studied. The chromatographic techniques included silica gel and reversed-phase (C_{18} RP) thin-layer chromatography (TLC) and C_{18} RP high-performance liquid chromatography (HPLC) using UV and mass spectrometric (MS) detection with atmospheric pressure chemical ionization (APCI). The TLC separation of the isomeric triterpenols lupeol, α-amyrin, β-amyrin and cycloartenol was achieved for the first time using C_{18} RP-HPTLC plates. Cycloartenol could be separated from related compounds only on C_{18} RP-TLC but not on the C_{18} RP-HPLC. δ-Amyrin isolated from the tomato fruit surface extract could be separated from other amyrins only by HPLC. Tandem mass spectrometry allowed discrimination between the isomers lupeol, α-amyrin, β-amyrin, δ-amyrin, cycloartenol and between lupeol acetate and cycloartenol acetate. The combination of 3 TLC methods and 2 HPLC methods enables qualitative determination of all 12 compounds and proves to be useful for the analysis of plant extracts. It is recommended that TLC screening on silica gel and C_{18} RP be performed before HPLC analysis.

1. Introduction

Triterpenoids represent a large class of secondary metabolites present in terrestrial and marine flora and fauna [1]. Their presence, especially in the plant kingdom, has aroused interest from both an evolutionary and a functional perspective. Besides compounds related to friedelin, the ursane and oleanane skeletons are the most commonly encountered among plant triterpenoids [2]. Their properties are of significant pharmacological importance: α-amyrin (ursane type) and β-amyrin (oleanane type) exhibit gastroprotective [3], antiprurutic [4] and hepatoprotective activity [5]. Oleanolic and ursolic acid with anti-inflammatory, hepatoprotective, antitumor and antimicrobial activities [6] are used commercially in cosmetics and health products.

Triterpenoids have been investigated by gravity-flow column liquid chromatography (GCC), thin-layer chromatography (TLC), gas chromatography (GC) and high-performance liquid chromatography (HPLC), each with its own characteristics [7]. GCC is appropriate as a preparative and semi-preparative technique [8]. GC with mass spectrometric detection [9–12] and/or flame ionization detection [12,13] and HPLC with UV detection [14–19] are the most commonly used techniques for detection and determination of triterpenoids. HPLC has also been used in combination with MS detection using a particle beam interface [20] and atmospheric pressure chemical ionization (APCI) [11,18,19]. TLC is a powerful tool especially for the screening of triterpenoids in plant extracts; the analyses are fast and simple because no sample pretreatment is required and large number of samples can be analyzed simultaneously. TLC on silica gel has been used for qualitative, as well as for quantitative [21–23] determination of α-amyrin [21,23] and lupeol [22]. There are reports about the separation of some triterpenoids with [7] or without [10,24] prior esterification on silver nitrate impregnated silica gel TLC/HPTLC plates. Lupeol, β-amyrin, taraxasterol and ψ-taraxasterol have been separated using chloroform–diethyl ether (19:1) [10], α-amyrin together with β-amyrin have been separated from lupeol using dichloromethane–ethyl acetate (24:1) [24], while lupeol acetate and β-amyrin acetate have been separated using chloroform–diethyl ether (97:3) [7]. The separation of oleanolic acid, β-amyrin and β-amyrin acetate on silica gel HPTLC plates was achieved using hexane–dichloromethane–methanol–water (4:5:0.9:0.1) [25]. Recently, a separation of ursolic acid and oleanolic acid was achieved after iodine derivatization on silica gel HPTLC plate using petroleum ether–ethyl acetate–acetone (8.2:1.8:0.1) [26]. However, there is no report of the TLC separation

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of isomeric cycloartenol, lupeol, α-amyrin, β-amyrin and of isomeric lupeol acetate and cycloartenol acetate without prechromatographic derivatization or impregnation of the silica gel.

Determination of triterpenoids in plant extracts is rather difficult since most plants contain various triterpenoid compounds with similar structures and polarities. That some of them are isomers renders the separation even more difficult. Moreover, triterpenoids lack chromophores and therefore the sensitivity of UV detection is rather low and the choice of the mobile phase is limited. Therefore, a combination of complementary chromatographic techniques such as TLC, GC with MS detection and HPLC with UV and MS(–MS) detection must be employed for the determination of triterpenoids in plant extracts. We have already reported the identification of lupeol (lupane skeleton), α-amyrin (ursane skeleton) and β-amyrin (oleanane skeleton) in the cabbage leaf surface extracts, using TLC on silica gel and HPLC with UV and APCI-MS(–MS) detection [18]. In the present work we have studied the applicability of TLC and HPLC to the separation of some ubiquitous triterpenoids and two chemically related sterols commonly present in plants. For this purpose we developed new TLC, HPLC-UV and HPLC–APCI-MS methods.

2. Experimental

2.1. Chemicals and standard solutions

All chemicals (except δ-amyrin) including solvents were at least of analytical grade. Ethyl acetate, dichloromethane, chloroform, *n*-hexane, methanol, *n*-propanol, isopropanol, tetrahydrofuran, acetone and acetonitrile (HPLC grade) were from Merck (Darm-

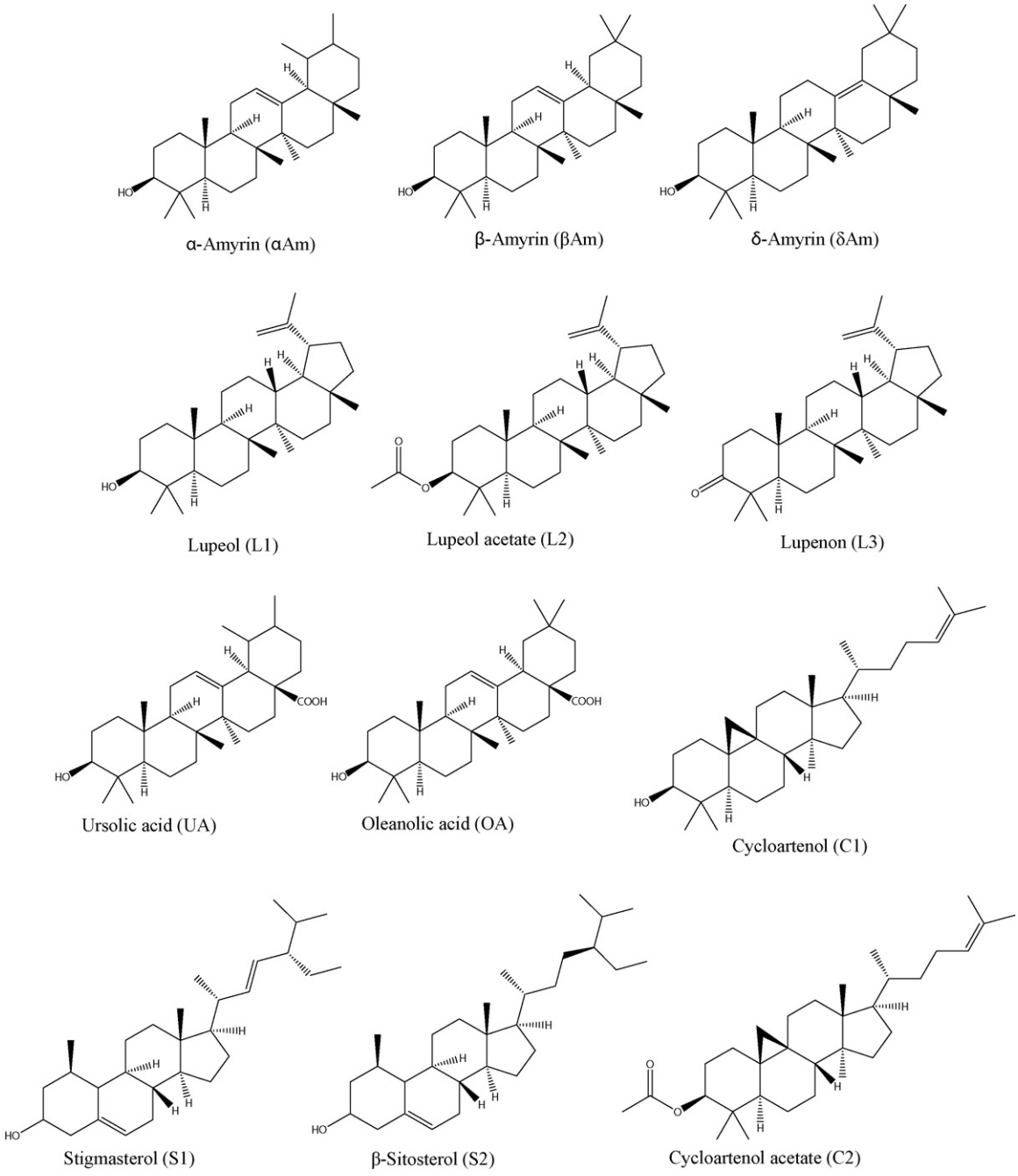

Fig. 1. Chemical structures of the studied compounds and abbreviations applied.