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Separation of a mixture of paraconic acids from *Cetraria islandica* (L.) Ach. employing a fluorous tag—catch and release strategy

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Abstract—A light-fluorous catch and release approach application has been designed to the separation of a mixture of three paraconic acids extracted from the Island moss (*Cetraria islandica* (L.) Ach.). The (+)-protolichesterinic acid was caught and released via a Michaël/retro-Michaël addition sequence with a fluorous thiol, while the resulting two other compounds were classically separated, allowing the isolation of (+)-roccellaric acid for the first time in this lichen.

(+)-Protolichesterinic acid (**1**),[1] one of the major secondary metabolites of the well known lichen *Cetraria islandica* (L.) Ach., is the main representative of the small class of paraconic acids.[2] These chiral trisubstituted γ-butyrolactones—bearing a carboxylic acid group in the β-position and a lipophilic alkyl chain in the γ-position (Fig. 1)—were exclusively isolated from fungi,[3] especially from lichens,[4] and exhibit relevant antitumor,[5] antibiotic[6] and anti-inflammatory[7] activities. However, at least two very closely structurally related paraconic acids are always extracted as a mixture from a lichen species and their separation using classical techniques constitutes a major drawback to their study.

In the last ten years, the use of light-fluorous versus solid-phase techniques has emerged as a powerful alternative strategy for the separation of reaction mixtures in synthetic organic disciplines.[8] Indeed, it combines homogeneous reaction media and convenient separation of fluorous-tagged compounds from non-fluorous ones using a fluorous solid phase extraction (FSPE) over fluorous silica gel.[9] Many light-fluorous components such as catalysts or reagents are now available, considerably expanding the field of light fluorous chemistry.[10]

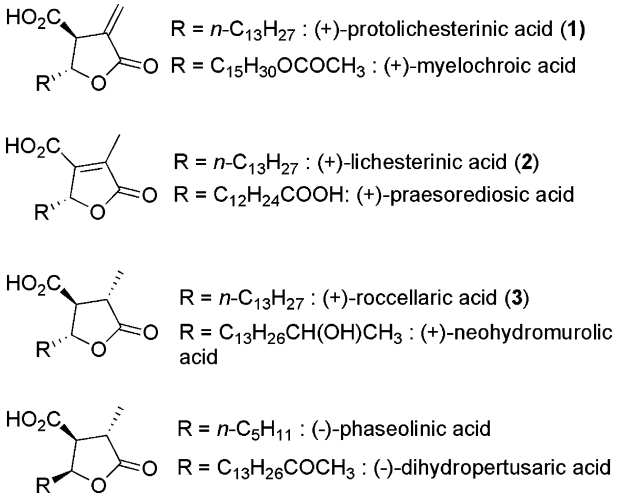

Figure 1. Structure of various paraconic acids.

Recently, fluorous scavengers[11] arose as efficient quenching reagents[12] or catch and release agents[13] in the synthesis of small organic molecules or isolation of peptides. We describe here a new application of the light-fluorous catch and release approach to the separation of a mixture of paraconic acids extracted from *C. islandica*, allowing us to isolate a minor compound from the lichen. To the best of our knowledge, this kind of approach has never been reported previously for the purification of natural products.

Keywords: Fluorous chemistry; Catch and release; Paraconic acids; Lichens.
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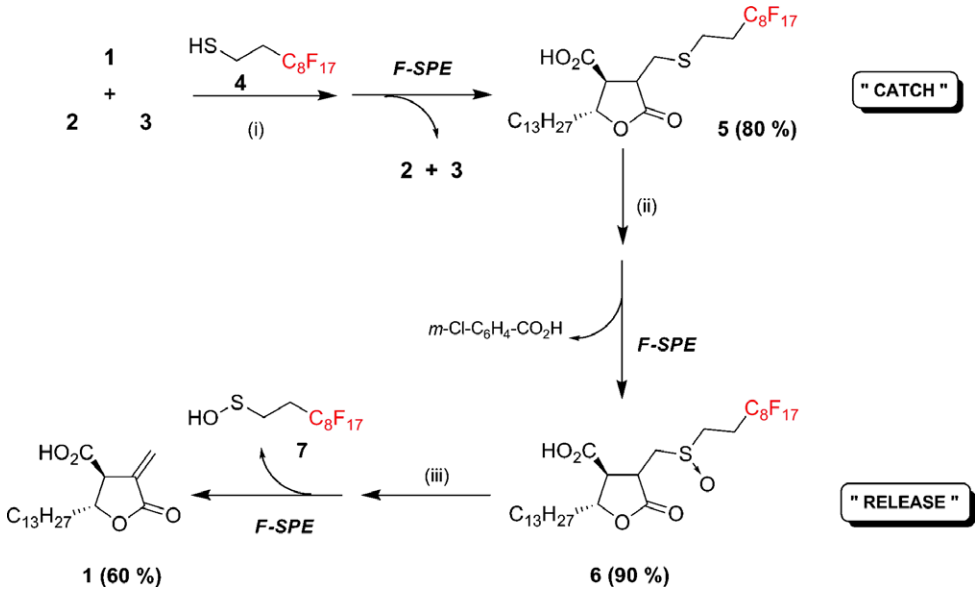
^{1}H NMR study of the precipitate obtained after cooling of the lipophilic extract (*n*-heptane) from a Danish specimen of *C. islandica* actually revealed the presence of (+)-protolichesterinic acid (**1**) and (+)-lichesterinic acid (**2**) (Fig. 1) as reported to date from the literature. In addition, we also noticed the presence of a third derivative identified as (+)-roccellaric acid (**3**)[14] (Fig. 1). This saturated protolichesterinic derivative, found in minute amounts, was however co-eluted with **1** in most of the TLC systems used. Therefore, to overcome the tedious chromatographic process required to separate these three paraconic acids, we investigated the performance of the light-fluorous technologies.

For the development of our catch and release strategy, the purpose was, in the first time, to catch with a fluorous tag and then release one of the compounds of this mixture in order to facilitate, in the second time, the separation of the other ones. The exocyclic double bond, only present on the (+)-protolichesterinic acid (**1**) in this mixture, seemed to be the most appropriate target. Therefore the fluorous tag had to be chosen regarding two aspects: (i) chemoselectivity towards this exocyclic double bond (*catch*) and (ii) easy removal of the tag at the end of the sequence for recovering **1** (*release*). Thus, the synthetic approach selected was the Michaël addition[15] of the commercially available fluorous thiol $CF_3(CF_2)_7CH_2CH_2SH$ (**4**)[16] on (+)-protolichesterinic acid (**1**) followed by adduct oxidation to sulfoxide and thermal release[17] of **1** (Scheme 1).

First, the mixture of **1**, **2** and **3** (in a molar ratio of 65/35/5, respectively) was stirred with the fluorous tag **4** in the presence of triethylamine in DMF during 2 h at room temperature[18] (disappearance of **1** was monitored by TLC) (Scheme 1). After acidification and removal of DMF, purification of the thioadduct (**5**) of (+)-protolichesterinic acid was conveniently carried out by FSPE. The crude reaction mixture was loaded onto a fluorous silica gel cartridge and lactones **2** and **3** were first eluted using a fluorophobic wash (MeOH and water in 90:10 ratio), whereas the fluorous thioether **5** was retained onto the SPE cartridge until elution with a fluorophilic solvent (CH_2Cl_2 and TFA in 95:5 ratio). It should be noticed that this thioadduct was scarcely soluble in all commonly used solvents, and addition of TFA revealed it necessary to completely solubilise it in usual solvents. We assume that intra and/or intermolecular hydrogen bonds between carboxylic acid group and sulfur atom, which can be disrupted in the presence of TFA, were involved. The fluorous derivative (**5**) of the targeted protolichesterinic acid (**1**) was hence efficiently separated from lactones **2** and **3** with a good yield of 80%, using a simple filtration on fluorous silica gel.

Second, recovering of **1** was achieved after an oxidation–elimination sequence. The subsequent oxidation of **5** by *m*-CPBA in THF at 0°C afforded the corresponding sulfoxide **6**. As previously described, FSPE allowed the purification of **6** in the fluorophilic wash with an excellent yield of 90%. It seems noteworthy—unlike solid-phase or polymer-supported catch and release methodologies—that all the fluorous intermediates could have been completely characterized by conventional analytical methods. The detagged α-methylene-γ-lactone **1** was finally regenerated with concomitant release of a fluorous sulfenic acid **7** through a thermal elimination in toluene. The residue was then purified by FSPE to afford **1** in the fluorophobic wash (MeOH and water in 80/:20 ratio) in a 60 % yield and complete removal of the fluorous product **7** in the fluorophilic wash (Et_2O).

With a 42% overall yield, the efficiency of this selective chemical 'extraction' was threefold higher than preparative TLC purification. Thus, the commercially selected



Scheme 1. Reagents and conditions: (i) **4** (1 equiv/**1**), NEt_3 (1.2 equiv/**1**+**2**+**3**), DMF, rt, 2 h; (ii) *m*-CPBA (1 equiv), THF, 0 °C, 2 h; (iii) toluene, reflux, 45 min.

fluorous thiol perfectly meets the requirements cited above, since spectral data and HPLC analysis confirmed that **1** was successfully recovered with an excellent purity of 99% after FSPE, and the catch step of the sequence was complete, affording a mixture of **2** and **3**, free of **1**.

Finally, this pre-purified mixture of **2** and **3** served as starting material for isolation of **2** and especially of **3** with preparative TLC. The (+)-roccellaric acid (**3**), which has only been isolated from *Roccellaria mollis* (Hampe) Zahlbr.,[14] was unambiguously identified by NOESY experiments and by comparison of their spectral data with those of the literature.[19] Thanks to this light-fluorous catch and release approach, it was isolated for the first time in *C. islandica*.

In summary, we have developed an original and efficient 'fluorous tag—catch and release' strategy for a straightforward isolation of the major (+)-protolichesterinic acid from an extract of Iceland moss, and the consequent separation of the two other close compounds **2** and **3**. Since paraconic acids are always described as a mixture in lichens, and their biogenesis[20] and chemotaxonomic distribution[21] are almost unknown, this methodology reveals to be a significant tool for further studies of these compounds. Furthermore, this approach may represent an attractive method to facilitate the isolation of minor secondary metabolites and would have potential for automated purification of different natural products in crude extracts, via the use of appropriately functionalized fluorous probes.

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Supplementary data

Experimental procedures and spectroscopic data. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2007.06.077.

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