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New and facile method of preparation of the anti-HIV-1 agent, 1,3-dicaffeoylquinic acid

Jiří Slanina,[a,*] Eva Táborská,[a] Hana Bochořáková,[a] Iva Slaninová,[b] Otakar Humpa,[c] W. Edward Robinson, Jr.[d] and Karl H. Schram[e]

[a]*Department of Biochemistry, Faculty of Medicine, Masaryk University, Komenského nám. 2, 662 43 Brno, Czech Republic*
[b]*Department of Biology, Faculty of Medicine, Masaryk University, Joštova 10, 662 43 Brno, Czech Republic*
[c]*Department of Organic Chemistry, Faculty of Science, Masaryk University, Kotlářská 2, 611 37 Brno, Czech Republic*
[d]*Department of Pathology and Microbiology and Molecular Genetics, University of California, Irvine, CA 92697-4800, USA*
[e]*Division of Medicinal Chemistry, Department of Pharmacology/Toxicology, College of Pharmacy, University of Arizona, Tucson, AZ 85721, USA*

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Abstract—A facile and inexpensive preparation of 1,3-dicaffeoylquinic acid (cynarin) from the leaves *Cynara cardunculus* L. (Asteraceae) without the use of any chromatographic steps is described. The procedure is based on separation of the fraction rich in 1,5-dicaffeoylquinic acid, isomerisation of 1,5-dicaffeoylquinic acid to cynarin and, owing to its higher polarity, the simple isolation of cynarin from the reaction mixture. Cynarin inhibited HIV-1 replication in MT-2 cell culture at non-toxic concentrations similar to other previously tested dicaffeoylquinic acids, which have been recently established as a potent and highly selective class of HIV-1 integrase inhibitors. © 2001 Elsevier Science Ltd. All rights reserved.

The plant polyphenolic compound 1,3-dicaffeoylquinic acid (**2**), otherwise known as cynarin, has been shown to have hypocholesterolemic,[1] hepatoprotective[2] and antioxidative[3] activity. The K^+-salt of **2** can induce a sweet taste of water and may provide an alternative to currently used sweeteners.[4] However, **2** is an artifact which can be formed from 1,5-dicaffeoylquinic acid (**1**) by intramolecular transesterification during the solvent extraction of plant material. Both **1** and **2** have been found only in plants of the family Asteraceae[5] and occur in some foods such as artichoke.[6]

Obtaining pure **2** from the mixture of mono- and dicaffeoylquinic acids in the crude extract of the plant material is problematic. The isolation of **2** from natural sources has been reported, but yields are low and the purity poor,[7] even following extensive chromatographic isolation.[8] While a synthetic method for **2** has been described,[9] the synthesis is uneconomical because of the high cost of the starting material and the low overall yield expected from the multistep reaction sequence.

Dicaffeoylquinic acids (DCQAs) have been recently established as a leading class of HIV-integrase inhibitors.[10] HIV-integrase is a virus-encoded enzyme that catalyses an essential step in the replication of HIV, insertion of viral DNA into the genome of the host cell. HIV-integrase is a promising target for the development of highly selective anti-HIV agents.[11] This new class of HIV-1 integrase inhibitors, DCQAs, inhibits strongly and irreversibly[10f] HIV-1 integration in biochemical assays and blocks viral replication at non-toxic concentrations in tissue culture.[10a,b,e,g] Recently, resistance to this new type of anti-HIV compounds was described.[12] Controversy over the mechanism of action of this class of inhibitors in vivo has recently arisen,[13] however, the potency of this class as inhibitors of integrase has been demonstrated by several laboratories. The use of HIV-1 integrase inhibitors together with already existing inhibitors for reverse transcriptase and HIV protease should be strongly synergetic.[14] We found that cynarin, like other DCQAs, is a potent and nontoxic inhibitor of HIV-1 replication in cells.

Reported herein is a simple and inexpensive method for the preparation of **2** from **1** without the isolation of **1** from the plant material and without use of any tedious chromatographic steps, which are normally required for the isolation of natural products from plant materials. Compound **2** can also serve as an easily obtainable

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* Corresponding author.

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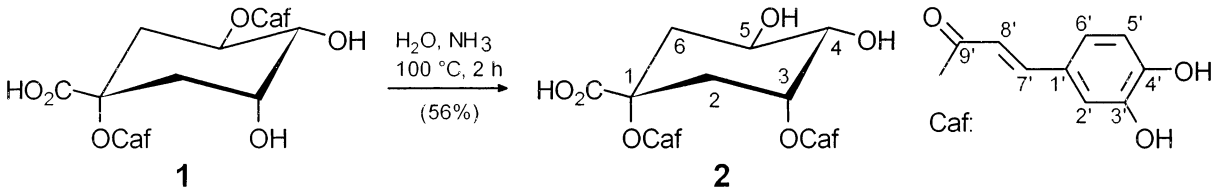

1 2

starting material for syntheses of compounds with improved anti-HIV activity.

1. Preparation of cynarin (2)

The dried leaves of *Cynara cardunculus* were extracted with methanol in a Soxhlet apparatus. An HPLC chromatogram of the crude extract displayed many minor peaks along with the peaks for the three main phenolic components, 5-caffeoylquinic acid (chlorogenic acid, **3**), 1,5-dicaffeoylquinic acid (**1**) and the flavone luteolin-7-glucoside (**4**). The methanolic extract was concentrated, diluted three times with water and extracted three times with petroleum ether to remove highly nonpolar compounds. The aqueous phase was acidified to pH 6 and extracted three times with diethyl ether. Extraction with diethyl ether was repeated after the aqueous phase had been gradually acidified to pH 5, 4, 3 and 2. The chromatograms of fractions extracted with diethyl ether between pH 3 and 5 contained one dominant peak corresponding to **1** with only a small amount of **3** present. (The pH 2 diethyl ether extract contained the largest amount of **3**, whilst **4** was completely insoluble in diethyl ether.) The residual **3** in the pH 3–5 diethyl ether extract was readily removed by washing with a small volume of water. The fractions rich in **1** were dissolved in water, made alkaline with dilute aqueous ammonia until the yellow colour of phenoxide was observed and the solution kept at 100°C for 2 h. Under these conditions **1** was smoothly converted to the significantly more polar **2**. The reaction mixture was then acidified to pH 4 and extracted twice with diethyl ether. The organic layer contained unreacted **1** and compounds with similar polarity as **1**, whereas **2,** owing to its higher polarity, remained in the aqueous phase. Compound **2** was obtained after concentration and crystallisation.

The purity of **2** was established by ^{1}H NMR and analytical HPLC (>97%) and supported by mass spectrometry. Additionally, the observed mp of **2**, 231–232°C, was about 3°C higher than the reported value,[7,8] reflecting the high purity of **2**. The overall yield of **2** was 0.11% calculated for the dry plant material and 20% calculated for **1** in the plant material.

The ^{1}H NMR spectra, including 2D NMR data, were internally consistent and corresponded closely to literature values.[15–17] Additionally, the long-range HMBC experiment revealed a clear crosspeak between one caffeoylic C-9′ and H-3 of the quinic acid residue.[17] Further evidence for esterification at C-1 and C-3 was provided by the downfield shift of these carbons in the ^{13}C NMR spectrum. The ^{13}C NMR spectrum of **2** has not been previously described.

^{13}C NMR of **2** (CD_3OD, 125 MHz): δ 33.05 (C-2), 41.36 (C-6), 67.89 (C-5), 73.04 (C-3), 75.39 (C-4), 81.21 (C-1), 174.64 (C-7), 115.24, 115.49 (2×C-8′), 115.55, 116.14 (2×C-2′), 116.64, 116.67 (2×C-5′), 122.10, 122.97 (2×C-6′), 127.53, 127.59 (2×C-1′), 146.54, 146.77 (2×C-3′), 147.20, 147.75 (2×C-7′), 149.31, 149.70 (2×C-4′), 167.88, 168.89 (2×C-9′).

Preliminary ESI MS and MS/MS experiments established the molecular weight of **2** to be 516 Da and the loss of two caffeic acid groups was evident.[18] Product ion spectra using MS/MS techniques showed the fragmentation of **2** to be considerably different from that of **1** and other DCQAs.[19]

2. Biological activity

Compound **2** inhibited HIV-1 replication in MT-2 cell culture with an EC_{50} value of 25 μM (for experimental details see Ref. 10b). The LD_{50} (50% inhibition of MT-2 cell growth) of **2** was 250 μM. In an additional experiment, compound **2** displayed low cytotoxicity against the HeLa cell line: **2** did not significantly alter the growth of the HeLa cell culture until 400 μM.

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