Efficient Isolation and Structure Analysis of (+)-Ranuncoside, a Unique Tricyclic Spiroacetal Glycoside, from Christmas Rose (Helleborus niger L.)

Eckehard Cuny and Franz-Dietrich Klingler

Abstract
The use of medicinal herbs as remedies reaches back to the Stone Age, and their importance as a source of drugs has continuously increased since then. Herbal ingredients can serve as active pharmaceuticals themselves or as lead substances for the development of synthetic pharmaceuticals with less toxicity, higher effectiveness or with new properties. To date, only 6% of the ∼600,000 plants on earth have been tested pharmacologically. Among these, the medicinal plant Helleborus niger L. (Christmas rose) is especially promising because its leaves contain (+)-ranuncoside 1, characterized by a spiroacetal ring system, a motif which is responsible for the biological activity of a multitude of natural products. Structure-activity relationship studies of (+)-ranuncoside 1 are lacking and no synthesis of 1 has been described yet. Therefore, we developed a protocol for the rapid and efficient isolation of 1 from the leaves of cultivated Christmas rose. Crystals of high purity were obtained that enabled us to study the stereochemistry of 1 by NMR spectroscopy in solution for the first time. The spiro configuration, the absolute stereochemistry, and the geometry of all three rings was then confirmed by x-ray structure analysis. Our data will enable future structure-activity relationship studies to assess the potential of 1 as a lead substance for the development of novel antibiotics and anticancer agents.

Keywords
spiroacetal, bioactivity, ranuncoside, medicinal herb, drug isolation, antibiotic, anticancer agent

Received: November 23rd, 2021; Accepted: December 9th, 2021.

Introduction
Plants were the first food of humans and it can be assumed that prehistoric people could differentiate between numerous edible and toxic plants and used medicinal herbs for healing purposes. Early discoveries of drugs in Europe reach back to the Stone Age, evidenced by a 5700 year old piece of chewed birch pitch from Betula pendula found in Denmark. Another example are two dried fragments of the polypore fungus Fomitopsis betulina presumed to be used for prehistoric medical purposes, which were found among the belongings of the 5300 year old glacier mummy named Ötzi the Iceman, discovered in 1991 in the Ötztal Alps near the Austrian–Italian border. Until the middle of the 18th century medicinal plants were the only source of organic drugs. Since then, the importance of medicinal plants has increased continuously. Around 50% of modern drugs depend on biogenic based compounds, and 10% of all drugs are pure medicinal plants. Helleborus species (Ranunculaceae) have been used as medicinal plants for over 2000 years, eg in ancient Greece, owing to their ingredients with potent pharmaceutical properties. Paracelsus, a seminal physician and alchemist of the 16th century, valued the leaves and roots of Helleborus niger L. (black hellebore or Christmas rose (Figure 1 [a]) as an effective remedy for numerous diseases. Even to this date, extracts of the rhizome and other subterranean parts of this plant are widely used in traditional medicine for a diverse range of symptoms and diseases. Helleborus niger extracts are also used in anthroposophic medicine, and clinical uses include the concomitant treatment of oncological diseases.

1Clemens-Schöpf-Institute of Organic Chemistry and Biochemistry, Darmstadt Technical University, Darmstadt, Germany
2Bio de tek GmbH, Griesheim, Germany

Corresponding Author:
Eckehard Cuny, Clemens-Schöpf-Institute of Organic Chemistry and Biochemistry, Darmstadt Technical University, Alarich-Weiss-Straße 4, 64287 Darmstadt, Germany.
Email: cuny@cuny.de

Creative Commons Non Commercial CC BY-NC: This article is distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 License (https://creativecommons.org/licenses/by-nc/4.0/) which permits non-commercial use, reproduction and distribution of the work without further permission provided the original work is attributed as specified on the SAGE and Open Access page (https://us.sagepub.com/en-us/nam/open-access-at-sage).
Helleborus niger extracts contain numerous active ingredients\textsuperscript{8,15-17} such as hellebrin\textsuperscript{18-21} and helleborine\textsuperscript{22,23}, which are powerful cardiac glycosides with pharmacological properties similar to those of the *Digitalis* glycosides.\textsuperscript{24-26} Moreover, a recent preclinical evaluation showed that black hellebore extracts are safe and have potential for cancer treatment.\textsuperscript{27} This finding was supported by two independent studies, which both found that the proliferation of tumor cells can be inhibited by *Helleborus niger* root and whole plant extracts.\textsuperscript{28,29}

The chemistry of the active ingredients in the above-ground parts of *Helleborus niger* has been investigated in a few studies. In 1973, Martinek\textsuperscript{30} isolated a crystalline compound from the ethanol extract of dried stems, leaves, and flowers of *Helleborus niger* L. which had been collected in the Austrian Alps (South Carinthia). In a subsequent paper by Martinek,\textsuperscript{31} the ingredient was identified as (+)-ranuncoside \textsuperscript{1} (structural formula: see [a] in Figure 1), due to its identical melting point and rotation with an isolated sample from *Ranunculus foetidus* L. and *Ranunculus repens* L.\textsuperscript{32,33} (Figure 1, [b] and [c], respectively).

Vitalini et al\textsuperscript{34} investigated the ingredients in the leaves of *Helleborus niger* L. as well, but with plants collected in the mountain woods of Valestino (Brescia, Italy). Interestingly, in contrast to Martinek, they did not find the highly crystalline (+)-ranuncoside \textsuperscript{1}, and instead isolated the following amorphous ingredients from the methanol extract: glucosyl-phenyllactic acid \textsuperscript{2}, but with undefined stereochemistry, and the flavonol glycosides \textsuperscript{3} and \textsuperscript{4}. The latter \textsuperscript{4} was also found in *Ranunculus chinen-sis* BGE.\textsuperscript{35} (structural formulas of natural products \textsuperscript{1-4}, see Figure 2). It is possible that Martinek and Vitalini et al collected leaves from different subspecies of *Helleborus niger* given that the subspecies *Helleborus niger* ssp. macranthus (Freyn) has a very restricted native range in Northern Italy and possibly parts of Slovenia.\textsuperscript{36} Alternatively, the variability in chemical composition of *Helleborus niger* ingredients might be due to environmental and climatic factors. Another study, based only on HPLC/MS-data, aimed to identify the ingredients of leaves and stems of *Helleborus niger* L. as well, but did not mention the ranuncoside \textsuperscript{1} either.\textsuperscript{37}

The spiroacetal ring system is responsible for the biological activity of a multitude of natural products.\textsuperscript{38-40} For that reason, we previously developed efficient and stereo-controlled routes to novel bicyclic motifs of spiroacetal natural products\textsuperscript{41,42}, as well as tricyclic spiroacetal domain derivatives of the plant glycoside ranuncoside.\textsuperscript{43} With regard to potentially novel antibi-otics and selective anticancer agents, we needed (+)-ranuncoside \textsuperscript{1} for further structure-activity relationship studies.

Since (a) a chemical synthesis of \textsuperscript{1} is not yet known, (b) an isolation from wild *Helleborus niger* specimens collected in their natural habitat of the Alps is not possible due to their protected status, and (c) a lack of access to plant material of *Helleborus foetidus* L. and *Ranunculus repens* L., coupled with difficult isolation and modest yield,\textsuperscript{31,32} we investigated for the first time the cultivated variant of Christmas rose which is cheap and easily accessible in large quantities from nurseries.

**Results and Discussion**

The history of isolation and structure clarification of ranuncoside \textsuperscript{1} have been extensively described by us in a previous study on spiroacetal domain derivative syntheses.\textsuperscript{43} Here, we describe for the first time the isolation of (+)-ranuncoside \textsuperscript{1} from cultivated Christmas rose (*Helleborus niger* L.). The procedure is easy to carry out, rapid, very efficient, and gives \textsuperscript{1} in good yield and in highly crystalline form.

**Isolation of (\textsuperscript{+})-Ranuncoside \textsuperscript{1} from Christmas Rose**

There are many different techniques available for extraction and isolation of natural products.\textsuperscript{44} Generally, the plant material has

---

**Figure 1.** The poisonous flowering plants (a) *Helleborus niger* L. (black hellebore, Christmas rose) and (b) *Helleborus foetidus* L. (stinking hellebore) are native to mountainous areas of Central and Southern Europe, and (c) *Ranunculus repens* L. (creeping buttercup) is native to Europe, Asia, and northwestern Africa. They all belong to the Ranunculaceae family and contain the spiroacetal glycoside (\textsuperscript{+})-ranuncoside \textsuperscript{1} (structure superimposed in yellow color). Photos (a) and (c) were taken by author E. C. in his garden in Seeheim, Germany (April 2020 and December 2019, respectively). Photo (b) was provided by Dr Schneckenburger, Botanical Garden, Darmstadt Technical University, Germany (April 1995).
to be pre-prepared carefully prior to extraction of phytochemicals. For a selective and efficient extraction of a polar substance like the glycoside \((+)-\text{ranuncoside 1}\) it is important to remove the nonpolar, “fatty” components first.

To achieve a defined solid plant material, the green parts (leaves, stems, flowers) of the plant were dried in a vacuum drying oven to a constant weight and ground to a fine powder. The drying is very important in order to achieve a deep penetration of the water immiscible solvent in the first step. The small particle size leads to a much higher surface area which is of crucial importance for the solid-liquid extraction efficiency. In order to remove the nonpolar compounds, we used classical Soxhlet extraction with chloroform (trichloromethane) as solvent, given that \((+)-\text{ranuncoside 1}\) is insoluble in this solvent. For small-scale solid-liquid extraction this semi-continuous technique is favorable because of its simplicity and efficiency. In 1998, Luque de Castro and García-Ayuso compared the performance of Soxhlet extraction of solid materials with other conventional extraction methods (maceration, use of ultrasound, and a combination of both) and new extraction techniques such as microwave-assisted processes (MAP), microwave-assisted solvent extraction (MASE), accelerated solvent extraction (ASE), and supercritical fluid extraction (SFE). They concluded that conventional Soxhlet extraction, which has been widely used worldwide for many decades, was superior to the other conventional and modern techniques due to its ease of use and efficiency. However, it has to be considered that exposure to heat may be the cause of artifact formation depending on the method used and the stability of the compounds of interest. In the case of the relatively stable compound \((+)-\text{ranuncoside 1}\) no degradation was observed by TLC-monitoring during all process steps.

After removing chlorophyll, carotenoids, and other nonpolar components, the Soxhlet extraction was simply continued by changing the solvent to an ethanol/water mixture. In this step, the \((+)-\text{ranuncoside 1}\) and some other compounds of similar polarity were extracted. To isolate the pure product, the crude residue, after removal of the ethanol/water solvent, was flash-chromatographed on silica with a gradient of dichloromethane/methanol and finally recrystallized from ethanol/water.

With this simple and efficient laboratory process we were able to extract over 500 mg of the spiroacetal-glycoside \((+)-\text{ranuncoside 1}\) for a complete structural analysis and for further research purposes. In the future, this method can be scaled up for the provision of even larger amounts of \((+)-\text{ranuncoside 1}\). Moreover, it should be relatively easy to develop a “green” extraction process based on the procedure described here, because ethanol and water are green solvents that are applicable to large scale processing.

Figure 2. Isolated natural products from the alcohol extract of dried leaves of christmas rose (Helleborus niger L.): \((+)-\text{ranuncoside 1},^{31} \text{glucosyl-phenyllactic acid 2, flavon glycoside 3, and kaempferol glycoside 4.}^{34}\)
Structural Elucidation of (+)-Ranuncoside 1

(+)-Ranuncoside 1 (structure, see Figures 2 and 3) is a spiro furan-dioxane-pyran tricycle with a complex substitution pattern and stereochemistry. The central dioxane ring is fused twofold with the pyranose moiety in trans-manner and is additionally linked to a saturated γ-lactone ring in spiroacetal manner. In total, 6 stereogenic centers are present in the molecule. The spiro carbon has (R)-configuration, while the other configurations in the pyran portion originate from D-glucose.

Systematic name and atomic numbering were made using SciFinder and ChemDraw. Confusingly, the name ranuncoside was, after the first naming by Tscheche et al., later also used for two entirely different structures: (a) pentacyclic triterpenoid glycosides from Hydrocotyle ranuncoloides (Apiaceae) and (b) an olefinic glycoside from Ranunculus muricatus (Ranunculaceae) with unsolved stereochemistry in the hexose moiety.

The structure of (+)-ranuncoside 1 has not yet been investigated by NMR spectroscopy. Here, we unambiguously confirm its complex structure using NMR and additional x-ray analysis (see also Experimental). In the 500 MHz proton NMR spectrum, measured in DMSO-d6 (see Figure 3), the 2 well separated pairs of shifts at high field, δ 2.02 / 2.09 ppm and δ 2.53 / 2.66 ppm, are particularly striking for the 2 methylene groups in the five-membered ring. By means of an HSQC spectrum the 2 protons of the pair δ 2.02 / 2.09 ppm and the pair δ 2.53 / 2.66 ppm are each located at different carbon atoms, C-3 (δ 28.77 ppm) and C-4 (δ 27.09 ppm). Due to the chemical shift to lower field by the carbonyl group, they can be clearly assigned as 3-C H2 (δ 2.02 / 2.09 ppm) and 4-C H2 (δ 2.53 / 2.66 ppm). The observed NOE interaction between 3′-Heq (4.02 ppm) and 3-HB (2.09 ppm) enables the exact allocation of all 4 protons (see stereo structure of 1 in Figure 3).

In the standard work “Stereochemistry of Carbohydrates” by Stoddart, the conformational analysis of furanoid rings is described in detail. Against this background, the observed large geminal coupling constants of 13.4 (3-CH2) and 17.5 Hz (4-CH2), the size of the remaining coupling constants of 3.5, 9.5, 9.6 and 9.8 Hz indicate a flattened half-chair conformation of the five-membered ring. In combination with the x-ray
structure analysis data (see below), a $^{3}T_{2}$ twist (half-chair) conformation of the furanoid ring is present in solution, too. In an additional Newman-projection formula, the arrangement of all 4 protons is clearly presented (see structures 5 and 6 in Figure 4).

As expected, the 2 protons of the methylene group in the central dioxane portion $3'-H_{as}$ and $3'-H_{eq}$ appear as 2 sharp doublets (3.78 and 4.02 ppm) and with large geminal coupling (12.7 Hz), respectively. Based on the smaller coupling constant of 7.3 Hz and the chemical shift at $\delta=4.38$ ppm, the other doublet could be assigned to the full acetal proton $4'a-H$ at the annulation position of dioxane and pyran ring. In terms of its NOE interaction with $3'-H_{as}$ and $3'-H_{eq}$, both dioxane protons at C-3' ($3'-H_{as}$ and $3'-H_{eq}$) can be assigned. From the pyran ring protons with usual stereochemistry of $\beta$-d-glucose only 7'-H can be assigned as a ddd at $\delta=3.14$ ppm, while the shifts of 6'-H, 8'-H and 8'a-H overlap at 3.27 ppm to a multiplet. This can be separated by adding D$_2$O to the measuring solution. As seen in the insert in the spectrum the 500 MHz $^1$H NMR spectrum simplifies very advantageously. Thereby, (a) couplings of the protons of the the OH-groups are eliminated and (b) the broad shift at $\delta=3.27$ ppm is split up and 8'a-H (dd, 3.23 ppm), 6'-H (dd, 3.28), and 8'-H (dd, 3.32 ppm) can be assigned unambiguously.

The appearances of the shifts at $\delta=3.46$ (6''-H$_{A}$) and $\delta=3.67$ ppm (6''-H$_{B}$) with 1H-ddd are both typical for the exocyclic 6''-CH$_2$OH group of the pyran ring. The shifts of the 4 hydroxyl groups are well separated: 2 near sharp doublets at $\delta=5.21$ (4.5 Hz) and $\delta=5.25$ (5.4 Hz) for 7''-OH and 8''-OH, the triplet at $\delta=4.70$ (5.7 Hz) for the 6''-CH$_2$OH

Figure 4. Partial structures 5, 6 and 7 illustrate the conformation of the five-membered furanoid part in (+)-ranuncoside 1 (highlighted in blue color). 5 shows the arrangement of the methylene protons in the half-chair conformation of the five-membered ring (the half-chair is flattened for clarity). The Newman projection 6 illustrates the approximate dihedral angles. Structure 7 depicts the torsions angles which are responsible for the half-chair conformation. The perspective drawings 8 and 9, as determined by x-ray crystallography analysis, show the 2 hydrogen bonds and the all-chair conformation of the trioxa transdecaline ring system with a $\beta$-D-glucose unit (structure 8) as well as the five-membered furanoid ring which is only slightly angled from the plane (structure 9).
group and the big sharp singulet at δ = 3.39 for 1 molecule of water.

The 13C NMR spectral data of (+)-ranuncoside 1 (see Experimental) are characterized by the deep field shift of C-5 (lacton carbonyl group) at δ = 175.55 ppm as well as 2 nearby shifts in the center of the spectrum: the quarternary spirocarbon C-2 (103.63 ppm) and the 1 proton bearing full acetal carbon C-4′a (96.91 ppm). At high field, the 4 shifts for the CH2 groups: C-4 [27.09], C-3 [28.77], 6′-CH2OH [60.62] and C-3′ [69.32] are found. The resonances of the remaining carbons of the pyran ring C-7′ [70.33], C-8′ [72.72], C-8′a [74.47] and C-6′ [78.66 ppm] are clearly assignable by a carbon proton correlation spectrum. For the complete 1H, 13C and 2D NMR spectra, see the Supplemental Material [Figure S1-S9].

The structural elucidation of (+)-ranuncoside 1 by x-ray analysis was published 50 years ago and was marked by the absence of structural formulas illustrating the conformation of 1. Since no stereochemistry data were provided, a stereochemical interpretation of 1 was not possible. In addition, the β-ᴅ-glucoside unit was drawn arbitrarily in the L-configuration. Because of this deficiency, we have subjected the crystals, isolated from cultivated Christmas rose, to an x-ray analysis again. The result is depicted in Figure 4 with a side and top view of (+)-ranuncoside 1. We identified 2 significant hydrogen bond bridges: (a) between the carbonyl oxygen atom of the five-membered ring and 1 molecule of crystal water and (b) between the hydroxyl group on C-8′ and the O-1′-dioxan ring oxygen atom. The attachment of the tetrahydrofuran ring in a spiroacetal manner with an axial arrangement of its oxygen atom to the trioxa decaline ring system was also clearly noticeable (Figure 4, structure 8). Anomeric stabilization effects are responsible for this arrangement and cause the R-configuration of (+)-ranuncoside 1 at the spiro center. Both six-membered rings, dioxan and pyran, of the trioxa trans-decaline frame work possess cyclohexan-type chair conformation. The β-ᴅ-glucose unit possesses an all-equatorial arrangement of the substituents, as expected.

For better clarity, in the structure of 9 (Figure 4) the hydrogen bonds are omitted. It shows the tetrahydrofuran ring with a nearly planar arrangement of the carbonyl group (C-5) and its neighbouring atoms (O-1 and C-4). The remaining two ring atoms (C-2 and C-3) are slightly angled from the plane as confirmed by the measured torsion angles which are given in the partial structure 7. In the course of the conformational analysis of the stereo structure of cyclopentanes, appropriate torsion angles yield the corresponding half-chair conformation.

**Conclusions**

Medicinal plants have a long medical-cultural tradition which reaches far back into human history. With the recent trend of returning to natural medicines, they are now again of great interest. The main reasons for this are (a) the cheap source of raw materials for drugs and (b) the vast amount of different ingredients which could serve as lead substances for the development of pharmaceuticals with lower toxicity, greater efficiency or with new properties. A candidate that meets both requirements is (+)-ranuncoside 1, which occurs in the medicinal plant Christmas rose (*Helleborus niger* L.). This natural product is particularly promising because it possesses a unique spirocetal ring system, which is responsible for the biological activity of a multitude of natural products. In the course of our research for potentially novel antibiotics and selective anticancer agents, we needed (+)-ranuncoside 1 for further structure-activity relationship studies.

Since the natural substance (+)-ranuncoside 1 had not been available, we here isolated it from the cultivated variant of the Christmas rose for the first time. The green parts of the plant were dried, ground to a fine powder and “defatted” by a classical Soxhlet extraction. From this material, the (+)-ranuncoside 1 was extracted by a simple change to ethanol/water and finally

---

**Table 1. Crystallographic Data for the Crystal Structure Determination of the Monohydrate of (+)-Ranuncoside 1.**

| Property                        | Value         |
|---------------------------------|---------------|
| Empirical formula               | C11H12O5 · H2O |
| Formula weight                  | 276.09 + 18.02 |
| Temperature                     | 293(2) K      |
| Wavelength                      | 0.71073 Å     |
| Crystal system, Space group     | Monoclinic, P2₁ |
| Unit cell dimensions            | a = 5.2929(5) Å, α = 90° |
|                                | b = 10.7971(1) Å, β = 95.73(1)° |
|                                | c = 11.408(1) Å, γ = 90° |
| Volume                          | 645.51(10) Å³ |
| Z                               | 2             |
| Density (calculated)            | 1.507 mg/m³   |
| Absorption coefficient          | 0.133 mm⁻¹    |
| F(000)                          | 312           |
| Crystal size                    | 0.240 × 0.180 × 0.180 mm⁻¹ |
| Theta range for data collection | 2.604 to 25.350° |
| Index ranges                    | -6 ≤ h ≤ 4, -13 ≤ k ≤ -12, -13 ≤ l ≤ 13 |
| Reflections collected           | 2319          |
| Independent reflections         | 1777 [R(int) = 0.0130] |
| Completeness to theta = 25.242° | 99.4%         |
| Absorption correction           | Semi-empirical from equivalents |
| Max. and min. transmission      | 1.00000 and 0.95932 |
| Refinement method               | Full-matrix least-squares on F² |
| Data / restraints / parameters  | 1777 / 6 / 196 |
| Goodness of fit on F²           | 1.086         |
| Final R indices [I > 2σ(I)]     | R1 = 0.0386, wR2 = 0.0600 |
| R indices (all Data)            | R1 = 0.0370, wR2 = 0.0631 |
| Absolute structure parameter    | 0.1(7)        |
| Largest diff. peak and hole     | 0.129 and -0.140 eÅ⁻³ |

The atomic coordinates, bond length, anisotropic displacement parameters, hydrogen coordinate, torsion angles, and hydrogen bonds have been published as a CSD Communication and has a DOI and full CCDC citation: DOI: 10.5517/ccdc.csd.c28sm50; CCDC Number: 2112872. It is publicly available through http://www.ccdc.cam.ac.uk/conts/retrieving.html [or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, UK; Fax: +44 1223/336 to 033; E-mail: deposit@ccdc.cam.ac.uk].
purified by flash chromatography and recrystallization. This efficient procedure delivered the natural product in good yield as a highly crystalline monohydrate.

(+)-Ranuncoside I is characterized by a central dioxan ring, which is linked on both sides: (a) as spiroacetal with a furanoid ring (saturated y-lactone) and (b) twofold in a trans-manner with a ß-d-glucose moiety. (+)-Ranuncoside I has 6 stereogenic centers from which 5 bear a heteroatom (oxygen). Here, we have clarified the complex stereochemistry of I unambiguously using 500 MHz NMR spectroscopy in combination with an x-ray analysis. In terms of structure-activity relationships, the conformation of the furanoid ring and of the trioxa decalin scaffold of I are of particular importance.

As shown by the observed NOE interaction between H-3′ax of the dioxan ring and H-4′ of the pyran ring the trioxa decalin ring system has cyclohexane-type all-chair conformation and trans-configuration. Thus, it is rigid, as in steroids with trans-decalin partial structure. The ß-d-glucose unit possesses an all-equatorial arrangement of the substituents as easily detectable by the large coupling constants around 9 Hz and by x-ray analysis.

Because of the precise knowledge of the structure and conformation of (+)-ranuncoside I that is now available, and in combination with its easy large-scale accessibility from cultivated Christmas rose, a targeted synthesis of potentially novel antibiotics and selective anticancer agents, is now possible.

**Experimental**

TLC was performed on POLYGRAM® SILG/UV254 (Macherey Nagel & Co.). Preparative chromatographic separations were carried out on columns with Merck silica gel 60 (40-63 μm). Melting points were determined on a Büchi Tottoli apparatus and are uncorrected. Specific optical rotations were determined on a Perkin-Elmer Polarimeter 241 in 1 dm cuvettes at a wavelength of 589 nm. NMR spectra were measured on a Bruker 300 MHz spectrometer with Avance-II console and BBO probe as well as 500 MHz spectrometer equipped with its easy large-scale accessibility from cultivated Christmas rose, a targeted synthesis of potentially novel antibiotics and selective anticancer agents, is now possible.

**Isolation of (+)-ranuncoside I from Christmas rose** (*Helleborus niger* L.): 266 g of the green superterrean parts (leaves, stems and flowers) of Christmas rose (*Helleborus niger* L.) in blooming state (January 2021), obtained from a local plant nursery, were dried at 50 °C in vacuo in a drying oven to a constant weight (44 g). The dry material was ground to a powder in a warping blender and charged into a thimble of ca. 45 mm diameter. It was extracted in a soxhlet extractor with 800 ml of chloroform for 20 h. After removal of the chloroform, the extraction was continued with 750 ml of an ethanol-water mixture (70/30 vol) for ca. 24 h. The ethanol-water solution was evaporated to dryness in a rotary evaporator (ca. 23 g). 40 g of silica gel (Merck 60, 0.04-0.063 mm) were added and again evaporated to a dry powder. This material was loaded on top of a chromatography column (7 cm width x 12 cm length; ca. 230 g silica gel) and chromatographed with a gradient of dichloromethane/ methanol (85/15 → 80/20). The product (*R*<sub>t</sub>=0.6 in CH<sub>2</sub>C<sub>2</sub>/MeOH 80/20) was isolated and then recrystallized from ethanol/water (80/20) and dried in vacuo over calcium chloride in a desiccator: 563 mg of colorless needles, m.p. 217 to 218 °C, [α]<sub>D</sub>= +53.8 (c = 0.5, EtOH/H<sub>2</sub>O, 1 : 1) and [α]<sub>D</sub>= +52.2 (c = 0.5, MeOH), respectively; literature:[1] 206 to 208 °C (needles from EtOH/H<sub>2</sub>O), [α]<sub>D</sub>= +40.2 (c = 0.5, EtOH/H<sub>2</sub>O).

TLC analysis confirmed that the mother liquor still contained considerable quantities of the product, but it was not isolated by a second chromatography.

**1H NMR** (500 MHz, DMSO-d<sub>6</sub>) δ 2.02 (ddd, 1H, 3′a-H), 2.09 (ddd, 1H, 3′a-H), 2.03 (ddd, 1H, 4′a-H), 2.66 (ddd, 1H, 4′-H), 3.14 (ddd, 1H, 7′-H), 3.27 (m, 3H, 6′-H, 8′-H, 8′-a-H), 3.35 (s, H<sub>2</sub>O), 3.46 and 3.67 (each 1H-ddd, 6′-H<sub>a</sub> and 6-H<sub>b</sub> of 6′-CH<sub>2</sub>OH), 3.77 (d, 1H, 3′-H<sub>a</sub>), 4.01 (d, 1H, 3′-H<sub>e</sub>q), 4.38 (d, 1H, 4′-a-H), 4.64 (pt, 1H, 6′-CH<sub>2</sub>OH), 5.17 (1H-d, 8′-OH), 5.14 (1H-d, 7′-OH).

Each of the shifts are indicated as s for singlet, d for doublet, t for triplet, q for quartet, sext for sextet, oct for octet, m for multiplet, br for broad and p for pseudo. Mass spectra were run on a Bruker Impact II spectrometer.
Acknowledgments
The author (EC) thanks Prof. Dr. Michael Régelin for the opportunity to work in his group, Ms. Sabine Foro for providing the x-ray data and Dr. Jörg Fohrer for the helpful discussion of the NMR spectra. The author (FDK) thanks Dr. Vibhuti Klingler-Dabral for providing the facilities for product isolation and proofreading of the manuscript.

Author Contributions
FDK performed the isolation of (+)-ranuncoside. Both authors performed the structural elucidation. EC prepared the figures and wrote the manuscript.

Declaration of Conflicting Interests
The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding
The author(s) received no financial support for the research, authorship and/or publication of this article.

Ethical Approval
Not applicable, because this article does not contain any studies with human or animal subjects.

Informed Consent
Not applicable, because this article does not contain any studies with human or animal subjects.

ORCID ID
Eckehard Cuny https://orcid.org/0000-0003-2077-9536

Trial Registration
Not applicable, because this article does not contain any clinical trials.

Supplemental material
Supplemental material for this article is available online.

References
1. Petrovska BB. Historical review of medicinal plants’ usage. Phong Ret. 2012;6(11):1-5. doi: 10.4103/0973-7847.95849
2. Philipson JD. Phytochemistry and medicinal plants. Phytochemistry. 2001;56(3):237-243. doi: 10.1016/S0031-9422(00)00456-8
3. Jensen TZT, Niemann J, Iversen KH, et al. A 5700 year-old human genome and oral microbiome from chewed birch pitch. Nat Commun. 2019;10(1):5520. doi:10.1038/s41467-019-13549-9
4. Pleszczyńska M, Lemieszek MK, Siwulski M, et al. Fomitopsis betulina (formerly Piptoporus betulinus); the Iceman’s Polypore fungus with modern biotechnological potential. World J Microbial Biotechnol. 2017;33(8):1-12. doi:10.1007/s11274-017-2247-0
5. Newman DJ, Cragg GM. Natural products as sources of new drugs over the nearly four decades from 01/1981 to 09/2019. J. Nat. Prod. 2020;83(3):770-803. doi.org/10.1021/acs.jnatprod.9b01285
6. Dörfler HP, Roselt G. Heilpfianzen. 5th ed. Urania Verlag: 1990, p. 7-10. ISBN 3-332-00112-4. Language: German.
7. Barroso MS. The hellebore, the plant beloved by the Greeks: the reasons behind a myth. Vesalius: acta internationales historiae medicinae. 2015;21(2):30-37.
8. Maior MC, Dobrotá C. Natural compounds with important medical potential found in Helleborus sp. Cent Eur J Biol. 2013;8(3):272-285. doi:10.2478/s11535-013-0129-x
9. Wilkens J. Die Heilkraft der Christrose. 2th ed. AT Verlag: 2016, p. 17-25. ISBN 978-03800-831-6. Language: German.
10. Fischer H. Helleborus im altertum und bei paracelkus. Schweizer Med Wochenschr. 1936;66:484. Language: German.
11. Duke JA, Bogenschutz-Godwin MJ, du Cellier J, Duke PAK. Handbook of Medicinal Herbs. 2th ed. CRC Press, Taylor & Francis Group; 2002. ISBN 978-0-8493-1284-7.
12. Alström G, Jansson S. Helleborusauer och helsborin. Deringer: 1937, p. 309-310. ISBN 3-8047-1466-8. Language: German.
13. Soldner G. Helleborus Niger. Der Merkurstab, Journal of Anthroposophic Medicine. 2010;63(6):508-517. Language: German.
14. Seifert G, Rutkowski S, Jesse P, et al. Anthroposophic supportive treatment in children with medulloblastoma receiving first-line therapy. J Pediatr Hematol Oncol. 2011;33(3):e105-e109.
15. Takeishi M, Ichinobe Y. Chemical components of Helleborus Niger L. Bull Department General Education, College of Science and Technology, Nihon University, 1978;24:15-21. Language: Japanese.
16. Müller MB, Bertrams J, Stintzing FC. Stability of protoanemonin in plant extracts from Helleborus Niger L. and Pulsatilla vulgaris mill. J. Pharmaceut Biomed Analysis. 2020;188:113370. doi.org/10.1016/j.jpba.2020.113370
17. Liedke S, Lorch E, Goedings P, Wichtl M. Isolation of helleborin and helleborin. Arch Pharm. 1936;251(1-4):154-183.
18. Karrer W. Hellebrin, a crystallized glycoside from the rhizome of Helleborus Niger. Helv Chim Acta. 1943;26(4):364-374.
19. Vesalius: acta internationales historiae medicinae. 1936;26(5):1353-1367. Language: German.
20. Wissner W, Kating H. Botanical and phytochemical investigations of species of the genus Helleborus growing in Europe and asian minor. III. Quantitative contents of hellebrin in plants of the natural biotops and in culture. Planta Med. 1974;26(4):364-374. Language German. doi:10.1055/s-0028-1099-401.
21. Isaac O. Process for the recovery of Hellebrin. Degussa AG, Frankfurt, Germany, DE2038110 A 1972-02-10. Language: German.
22. Sieburg E. Helleborein. Arch Pharm. 1936;259(10):1016/j.jpba.2020.113370
23. Thäter K. Glucosides contained in the root of Helleborus Niger: helleborein and helleborin. Arch Pharm. 1897;235(6-7):414-424. Language: German.
24. Chen KK, Henderson FG, Anderson RC. The cardiac action of helleborus glycosides and their aglycones. J Pharm Exp Ther. 1950;99(4):395-400.

25. Meyer FOW. Industrial processes for the manufacture of cardia- cetotoxic products. IV. Convallaria majalis, Helleborus Niger, and Nerium oleander. Pharmazie. 1949;4(12):578-482. Language: German. doi:10.24355/dbbs.084-201902081440-0.

26. Loffler W, Essellier AF, Pedrazzini A. Treatment of cardiac disor- ders with hellebrin, a glucoside of helleborine. Schweiz Med Wochenschr. 1948;78:1021-1025. Language: French.

27. Felenda JE, Tureck C, Mörbit N, Herrick A, Müller MB, Stintzing FC. Preclinical evaluation of safety and potential of black hellebore extracts for cancer treatment. BMC Complement Altern Med. 2019;19(1):105. doi:10.1186/s12906-019-2517-5

28. Jesse P, Mottke G, Eberle J, Seifert G, Henze G, Prokop A. Apoptosis-inducing activity of Helleborus Niger in ALL and AML. Pediatr Blood Cancer. 2009;52(4):464-469. doi:10.1002/pbc.21905

29. Yokosuka A, Inomata M, Yoshizawa Y, Iguchi T, Mimaki Y. Bufadienolides and cycloed steroids from the whole plants of Helleborus Niger and their cytotoxicity. J Nat Med. 2021;75:393 to 402. doi:10.1007/s11418-021-01481-6

30. Martinek A. An unknown glycoside from dried leaves of Helleborus Niger. Planta Med. 1973;24(5):73-82. Language: German. doi:10.1055/s-0028-1099472.

31. Martinek A. Ranuncoside in dried stems, leaves and Helleborus Niger. Planta Med. 1974;26(7):218-224. Language: German. doi:10.1055/s-0028-1099380.

32. Tschecche R, Welmar K, Wulf G, Snatzke G. Glycosides with lactone-forming aglycone. VI. Subsequent products of a still unknown genuine precursor of ranunculin in Ranunculaceae. Chem Ber. 1972;105(1):290-300. Language: German. doi:10.1002/chem.19721050131.

33. Mariecurrena RA, Rasmussen SE, Lam J, Wollenweber E. X-Ray structure determination of a naturally occurring γ-lactone glucoside from Helleborus foetida L. Tetrahedron Lett. 1972;13(30):3091-3092. doi:10.1016/S0040-4039(01)85016-4

34. Vitalini S, Braca A, Fico G. Study on secondary metabolite content of Helleborus Niger L. Leaves. Fitoterapia. 2011;82(2):152-154. doi:10.1016/j.jfito.200400457

35. Zhou YP, Tan CH, Wang BD, Jiang SH, Zhu DY. Flavanoid glycosides from Rannunus chinensis BGE. Helv Chim Acta. 2007;90(10):1940-1945. doi:10.1002/hlca.200790202

36. Petričić Tarle D, Knezevic E. Helleborus chemotypes from yugoslav- ia recognizable by hellebrin contents. Acta Pharm Jugosl. 1977;27- (3):127-129.

37. Duckstein SM, Stintzing FC. Comprehensive study of the phenolics and saponins from Helleborus Niger L., leaves and stems by liquid chromatography/tandem mass spectrometry. Chem Biodivers. 2014;11(2):276-298. doi:10.1002/cbdv.201300267

38. Perron F, Albizati KF. Chemistry of spiroketals. Chem Rev. 1989;89(7):1617-1661. doi:10.1021/cr00097a015

39. Aho JE, Pihko PM, Rissa TK. Nonanomeric spiroketal in natural products: structure, sources, and synthetic strategies. Chem Rev. 2005;105(12):4406-4440. doi:10.1021/cr050559n

40. Sperry J, Wilson ZE, Rathwell DCK, Brimble MA. Isolation, bio- logical activity and synthesis of benzannulated spiroketal natural products. Nat Prod Rep. 2010;27(8):1117-1137. doi:10.1039/ B911514P

41. Cuny F, Lichtenhalter FW, Lindner HJ. Pyranol[d]-dioxanes through bisacetalic annulation of 2-ketosugars to glycol. Eur J Org Chem. 2004;2004(23):4901-4910. doi:10.1002/ejoc.200400458

42. Cuny F. Stereoselective synthesis of 1,6,9-trioxaspiro[4,5]decanes from D-glucose: novel structural motifs of spiroacetel natural products. Nat Prod Comm. 2020;15(4):1-12. doi: 10.1177/ 1934578X20909175

43. Cuny F. Stereoselective synthesis of spiroacetal domain derivatives of the plant glycoside ranuncoside, and of okadaic acid and dino- physisotoxins –1 and –2 from marine Algae. Nat Prod Comm. 2020;5(1):1-11. doi:10.1177/1934578X20971150

44. Altemimi A, Lakshassani N, Baharlonei A, Watson DG, Lightfood DA. Phytochemicals: extraction, isolation, and identification of bioactive compounds from plant extracts. Plants. 2017;6(4):42- 65. doi: 10.3390/plants6040042

45. Luque de Castro MD, García-Ayuso LE. Soxhlet extraction of solid materials: an outdated technique with a promising innovative future. Analyst Chim Acta. 1998;369(1-2):1-10. doi:10.1016/S0003-2670(98)00233-5

46. Ameer K, Shabaz HM, Kwon JH. Green extraction methods for polyphenols from plant matrices and their byproduct: a review. Compr Rev Food Science & Food Safety. 2017;16(2):295-315. doi: 10.1111/1541-4327.12253

47. Corsaro MM, Greca MD, Fiorentino A, Monaco P, Previtera L. Ranuncoside VII-A new oleane glycoside from Hydrocotyle ranun- colides. Nat Prod Lett. 1995;6(2):95-102. doi:10.1080/ 1057563950804406

48. Raziq N, Saeed M, Ali MS, Zafar S, Shahid M, Lateef M. A new glycoidic antioxidant from Ranunculus muricatus L. (Ranunculaceae) exhibited lipooxygenase and xanthine oxidase inhibition properties. Nat Prod Res. 2016;31(11):1251-1257. doi:10.1080/1111/1541-4327.12253

49. Stoddart JF. Stereochemistry of Carbohydrates. Wiley-Interscience, 1971, p. 97. ISBN 0-471-82650-2.

50. Mariecurrena RA, Rasmussen SE. The crystal structure of a naturally occurring γ-lactone glucoside (C11H16O8·H2O) from Helleborus foetida L. Acta Cryst. 1973;B29(5):1030-1035. doi.org/ 10.1107/S0567740873003808

51. Quinkert G, Egert E, Griesinge C. Aspekte der Organischen Chemie: Struktur. Basel. Verlag Helvetica Chimica Acta, VCH, 1995, p. 111. ISBN 3-906390-11-X. Language: German.

52. Gottlieb HE, Kostlyar V, Nuclelman A. NMR Chemical shifts of common laboratory solvents as trace impurities. J Org Chem. 1997;62(21):7512-7515. doi.org/10.1021/jo971176v.