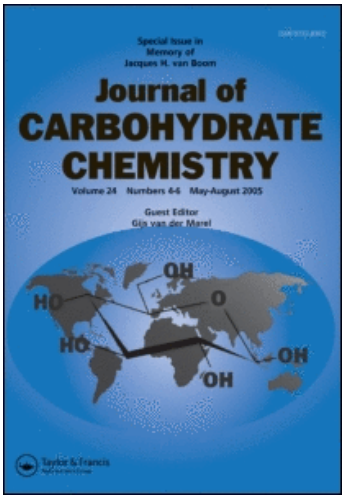


Journal of Carbohydrate Chemistry

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713617200

A Convenient Procedure for the Sulfation of Flavonoid Glycosides: Preparation of Rotin Nona- and Deca Sulfates

Vijay Nair[a]; Joseph P. Joseph[a]; John F. Poletto[a]; Seymour Bernstein[a]
[a] Medical Research Division, American Cyanamid Company, Pearl River, NY

To cite this Article Nair, Vijay , Joseph, Joseph P. , Poletto, John F. and Bernstein, Seymour(1987) 'A Convenient Procedure for the Sulfation of Flavonoid Glycosides: Preparation of Rotin Nona- and Deca Sulfates', Journal of Carbohydrate Chemistry, 6: 4, 639 — 644
To link to this Article: DOI: 10.1080/07328308708058893
URL: http://dx.doi.org/10.1080/07328308708058893

J. CARBOHYDRATE CHEMISTRY, 6(4), 639-644 (1987)

A CONVENIENT PROCEDURE FOR THE SULFATION OF FLAVONOID GLYCOSIDES: PREPARATION OF RUTIN NONA- AND DECA SULFATES

Vijay Nair*, Joseph P. Joseph, John F. Poletto, and Seymour Bernstein

Medical Research Division, American Cyanamid Company
Pearl River, NY 10965

Received January 12, 1987 - Final Form July 30, 1987

ABSTRACT

A satisfactory procedure for the sulfation of flavonoid glycosides is described and is illustrated by the preparation of rutin nona and deca sulfates. Compounds of this type are important modulators of the complement part of the immune system.

INTRODUCTION

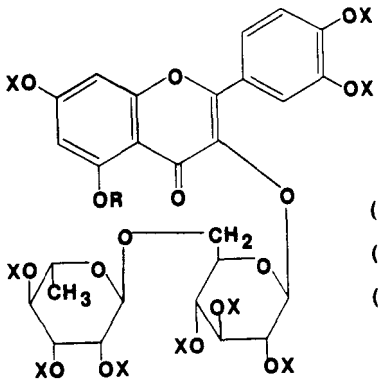
In connection with a program directed towards the discovery of synthetic modulators of the complement part of the immune system,[1-4] it became necessary to synthesize several glycoside sulfates. The sulfation of polysaccharides has been thoroughly investigated and reviewed[5] and there are isolated examples of the sulfation of certain aryl glycosides.[5,6] However, virtually no information is available on the sulfation of flavonoid glycosides. Although a variety of sulfur trioxide complexes have been used for sulfation, the more commonly used reagents are pyridine-sulfur trioxide and trimethylamine-sulfur trioxide complexes, presumably because of the stability and commercial availability of these reagents.

In our studies, the sulfation of flavonoid glycosides using either pyridine-sulfur trioxide or trimethylamine-sulfur trioxide led to complex mixtures of products. As a result of extensive investigations, we

have found that the use of triethylamine-sulfur trioxide under carefully controlled conditions leads to well defined and pure reaction products. The advantages of using triethylamine-sulfur trioxide for the sulfation of steroidal alcohols has been previously documented.[6] However, the reagent has not found wide use.

RESULTS AND DISCUSSION

We now describe a satisfactory procedure for the sulfation of flavonoid glycosides as illustrated by the sulfation of rutin (1) leading to the sodium salts of rutin deca (2) and nona (3) sulfates. As already mentioned, the use of triethylamine-sulfur trioxide is crucial for the success of the reaction. Carefully controlled work-up conditions are also important in the syntheses of 2 and 3.



(1) X = R = H

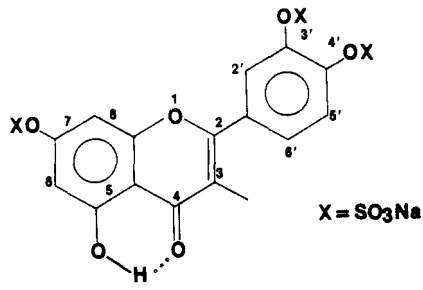
(2) X = R = SO_3Na

(3) X = SO_3Na, R = H

Rutin deca (H-sulfate) (2) has a propensity to undergo selective hydrolysis of the sulfate group at C-5 of the flavonoid moiety under acidic conditions. Therefore, it was necessary to maintain basic conditions during work-up of the triethylamine salt and its conversion to the sodium salt. Addition of triethylamine to the reaction-mixture prior to work-up was found to be a satisfactory way of controlling the pH. The work-up of the reaction-mixture without the addition of triethylamine afforded the nona (H-sulfate) sodium salt (3).

While reverse phase HPLC under ion pairing conditions enabled the detection and estimation of the purities of 2 and 3, ^{1}H and ^{13}C NMR spectra were instrumental in establishing the structures of these compounds. In general, the ^{1}H and ^{13}C resonance signals in both 2 and 3

were shifted downfield from those of rutin. Such chemical shifts are in keeping with the presence of highly electronegative sulfate substituents on the molecules. The signals due to protons on C-6 and C-8 in 2 and 3 were discernible due to their characteristic m-coupling. In 2 these signals appeared at δ 7.27 and δ 7.13, respectively. The corresponding protons in 3 resonated at δ 6.90 and δ 6.54. The difference in the chemical shifts for these protons in the two compounds suggested that 2 was sulfated at both C-5 and C-7 whereas the C-5 hydroxyl group was unsulfated in 3. Additionally the spectrum of 3 revealed a signal at δ 12.55 characteristic of a proton participating in a hydrogen bond to a carbonyl group in systems such as this.[7] A comparison of the ^{13}C NMR spectra of 2 and 3 provided further support for the assigned structures. In particular, the resonance position of the carbonyl carbon atom in these compounds provided compelling evidence for the assignment. In 2 this signal appeared at 175 ppm, whereas in 3 it resonated at 179.9 ppm. The downfield shift in 3 can only be attributed to hydrogen bonding of the hydroxyl group at 5 with the carbonyl group at 4 (see partial structure (3a) below).



(3a)

Such a downfield shift of the C=O carbon signal due to hydrogen bonding is well documented in flavonoids containing a C-5 hydroxyl group.[7,8] The above data along with other analytical results clearly indicated that 2 and 3 are the deca and nona sodium salts, respectively. It was interesting to note that after the addition of a drop of trifluoroacetic acid to a solution of 2, its ^{1}H and ^{13}C NMR spectra became indistinguishable from those of 3.

Both 2 and 3 were shown to be potent inhibitors of the complement system and the results of these biological studies will be published elsewhere. The procedure described herein for the sulfation of rutin can be applied to flavonoid glycosides in general.

EXPERIMENTAL

The triethylamine-sulfur trioxide complex was prepared by the reaction of triethylamine and chlorosulfonic acid by a procedure developed by us.[10] The rutin used was 'purum' grade (Fluka) and was dried *in vacuo* over phosphorus pentoxide. HPLC analysis was carried out using reverse-phase HPLC (C_{18} column, Waters Associates) under ion-pairing conditions. HPLC grade acetonitrile and specially purified water were used as the solvents. Tetrabutylammonium phosphate (PicA^R) was used as the ion-pairing reagent. The ^1H NMR and ^{13}C NMR spectra were obtained on Nicolet 100 MHz and 80 MHz instruments.

Rutin decakis (H-sulfate) Sodium Salt (2). The triethylamine sulfur trioxide complex (70.2 g, 0.388 mol) was dissolved in dimethyl-acetamide (245 mL) and Drierite (49 g) was added to it. The mixture was heated at 63-65 °C for 20 min. Rutin (5.95 g, 9.75 mmol) was added to the mixture with swirling. Heating was continued at 63-65 °C for 3 h. The reaction-mixture was then rapidly cooled and filtered into acetone (2.5 L) containing triethylamine (15 mL). The oil that separated was allowed to settle overnight at ~0 °C. The supernatent liquid was decanted and the oil washed several times with acetone to remove any excess triethylamine-sulfur trioxide complex.

The oily product was dissolved by the addition of 30% sodium acetate solution (40 mL) and water (50 mL). The solution was allowed to stand at room temperature for 20 min and then filtered. The filtrate was poured slowly into ethanol (2.3 L) with vigorous stirring. The stirring was continued for 40 min. The granular product was filtered, washed repeatedly with ethanol, and then with ether. It was then dried *in vacuo* at room temperature for 18 h. An off-white powder (14.5 g, 91%) was obtained. Anal. Calcd for $C_{27}H_{20}O_{46}Na_{10}S_{10}$, 4.4 H_2O, 2.7 Et-OH, 2.3 Na_2SO_4: C, 18.05; H, 2.12; S, 18.22; Na, 15.50. Found: C, 18.06; H, 1.88; S, 18.08; Na, 15.52. The following determinations supplement the above analysis. H_2O (KF)[11] = 3.6%, Et-OH (glc)[12] = 3.0%, Na_2SO_4 (non aq. titration)[13] = 9%. HPLC: Retention time 10.8 min (conditions: water-acetonitrile 55:45, PicA^R, flow rate 1 mL/min; 268 nm; chart speed 2"/min). UV: maxima at 313 nm, 253 nm. Infrared: 1622 cm^{-1}. ^1H NMR (DMSO-d6): δ 1.5 (3H, d, J = 6 Hz, CH_3), 3.75-4.7 (8H, multiple signals due to the disaccharide protons, individually unassignable), 5.16 (1H, d, J = 4.5 Hz, anomeric proton of rhamnose moiety), 5.91 (1H, d, J = 5.8 Hz, anomeric proton of glucose moiety), 7.13 (1H, d, J = 2 Hz, H-8), 7.27 (1H, d, J = 4 Hz, H-6), 7.66 (1H, d, J

= 4 Hz, H-5), 8.21 (1H, 5, H-2'). 8.25 (1H, d, J = 4Hz, H-6[1]). ^{13}C NMR (DMSO-d_6): δ 9.1 (rhamnose methyl), 157.4 (C-7), 158.0 (C-5), 175.0 (C=O).

Rutin nonakis (H-sulfate) Sodium Salt (3). The triethylamine-sulfur trioxide complex (70.2 g, 0.388 mol) was dissolved in dimethylacetamide (245 mL) and Drierite (49 g) was added to it. The mixture was heated at 63-65 °C for 20 min. Rutin (5.95 g, 9.75 mmol) was added to the mixture with swirling and the heating was continued at 63-65 °C for 8 h. The reaction mixture was then rapidly cooled and filtered into acetone (2.5 L). The oil that separated was allowed to settle overnight at room temperature. The supernatent liquid was decanted and the oil washed several times with acetone to remove any excess triethylamine-sulfur trioxide complex.

The oily product was dissolved by the addition of 30% sodium acetate solution (40 mL) and distilled water (50 mL). The solution was allowed to stand at room temperature for 20 min and then filtered. The filtrate was added gradually into ethanol (2.3 L) with vigorous stirring. The stirring was continued for 40 min. The granular product was filtered, washed repeatedly with ethanol and then with ether. The product was then dried in vacuo at room temperature for 18 h. A yellow powder (12.8 g, 86%) was obtained. Anal. Calcd for $C_{27}H_{21}O_{43}Na_9S_9 \cdot 3\ H_2O$, 3 Et-OH, $3Na_2SO_4$: C, 18.46; H, 2.11; S, 17.22; Na, 15.50. Found: C, 18.35; H, 1.88; S, 17.32; Na, 16.31. Also found: H_2O (KF)[11] = 3%, Na_2SO_4 (non-aq. titration)[13] = ~10%. HPLC: Retention time, 11.5 min (water acetonitrile 55:45, PicA^R, flow rate 1 mL/min; 268 nm, chart speed 2"/min). UV: maximum at 265 nm. Infrared: 1648, 1605 cm^{-1}. ^{1}H NMR (DMSO-d_6): δ 1.29 (3H, d, J = 6.2 Hz CH_3), 3.5-4.8 (8H, multiple signals due to the disaccharide protons, individually unassignable), 5.1 (1H, d, J = 3.2 Hz, anomeric proton of rhamnose), 5.91 (1H, d, J = 5.0 Hz, anomeric proton of glucose). 6.54 (1H, d, J = 2 Hz, 6), 6.90 (1H, d, J = 2 Hz, 8), 7.68 (1H, d, J = 4 Hz, 5'), 8.06 (1H, d, J = 4 Hz, 6'), 8.14 (1H, s, 2'), 12.55 (1H, s, 5). ^{13}C NMR (DMSO-d_6): δ 98.5 (C-8), 100 (C-6), 102 (C-9), 136.1 (C-2, 143.6 (C-3'). 146.7 (C-4'), 157.1 (C-5'), 157.8 (C-6'), 158.6 (C-7), 160-9 (C-5), 179.9 (C=O).

ACKNOWLEDGEMENTS

We wish to thank Dr. Martin J. Weiss for his interest, Mr. George O. Morton for the ^{1}H and ^{13}C NMR spectra, and Mr. L. M. Brancone and his associates for the elemental analysis.

REFERENCES AND NOTES

1. A. G. Osler, "Complement", Prentice Hall, Englewood, NJ, 1976.

2. I. Gigli, Clin. Exptl. Dermatology 4, 271 (1979).

3. R. A. Patrick and R. E. Johnson, Reports Med. Chem., 15, 193 (1980).

4. N. R. Cooper, Adv. Immunology, 37, 151 (1985).

5. K. B. Guisley, in Carbohydrate Sulfates, R. G. Schweiger, Ed.; (ACS Symposium Series, 77, 1978) and references cited therein.

6. J. P. Dusza, J. P. Joseph and S. Bernstein, Steroids, 12, 49 (1968) and references cited therein.

7. K. R. Markham and T. J. Mabry in The Flavonoids, J. Harborne and H. Mabry, Eds.; Chapter 2, Academic Press, New York, NY, 1975, p 64.

8. E. Wenkert and H. Gottlieb, Phytochemistry, 16, 1811 (1977).

9. V. Nair and S. Bernstein, Unpublished results.

10. V. Nair and S. Bernstein, Org. Prep. Proced. Internat., 19, 34 (1987).

11. Karl-Fischer titration to determine the water content was carried out by the modified procedure developed by Scholz. See E. Scholz and Z. Fresenius, Anal. Chem., 303, 203 (1980).

12. The ethanol content was determined by routine gas chromatographic analysis of a solution of the compound in dimethyl formamide using a glass column (zone temp. ~ 150 °C).

13. The sodium sulfate was determined by a non-aqueous titration procedure. See: E. P. Serjeant, Potentiometry and Potentiometric Titrations, John Wiley and Sons, New York, NY, 1984, p 638 and references cited therein.