Nigellidine-4-*O*-sulfite, the First Sulfated Indazole-Type Alkaloid from the Seeds of *Nigella sativa*

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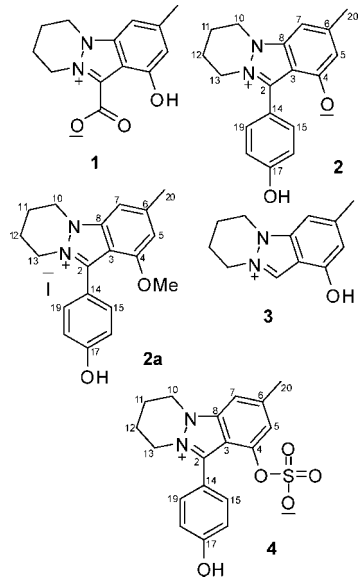
The rare indazole-type alkaloid nigellidine (**2**) is accompanied by its 4-*O*-sulfite (**4**) in the seeds of *Nigella sativa*. Compound **4** may represent the true natural product leading to nigellidine (**2**) via hydrolysis of the sulfate functionality during the isolation process. The structure of nigellidine-4-*O*-sulfite (**4**) is confirmed by NMR, MS, and X-ray crystallographic data. This is the first report of the natural occurrence of sulfated indazole-type alkaloids.

Nigella sativa Linn. (Ranunculaceae), known as black cumin, is an herbaceous plant that grows in Mediterranean countries, and its seeds are used as a spice. *N. sativa* has been used for centuries in Middle Eastern folk medicine for lactation deficiency, diabetes, hypertension, and cardiac and sexual diseases.[1–3] Owing to the pharmacological significance of black cumin, its phytochemical investigation was undertaken as part of our program to identify compounds that may be used as chemical or biological markers in dietary supplements.



Naturally occurring alkaloids containing the indazole nucleus are rare and confined to only three analogues. These compounds comprise nigellicine (**1**) and nigellidine (**2**) from *N. sativa*[4,5] and nigeglanine (**3**) from *Nigella glandulifera* Freyn *et* Sint.[6] Our continued investigation of *N. sativa* has led to identification of the 4-*O*-sulfite[7] (**4**) of nigellidine. Compound **4** is possibly the true natural product leading to nigellidine (**2**) via hydrolysis of the sulfate moiety during the isolation process. In contrast to the natural abundance of steroidal glycoside sulfates, sulfated alkaloids are rare,[8,9] and **4** indeed represents the first naturally occurring indazole-type alkaloid. Herein we also present X-ray crystallographic data

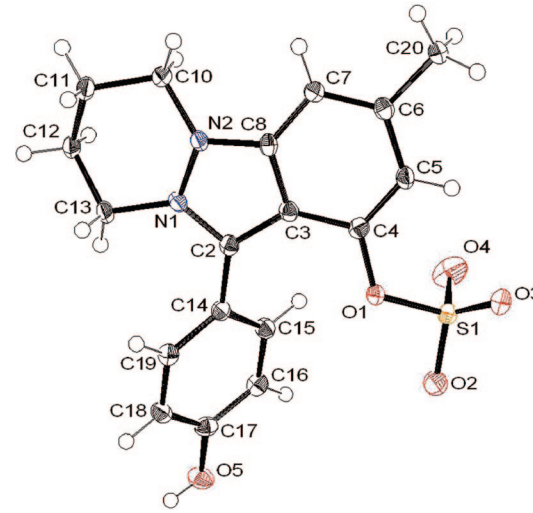


Figure 1. ORTEP drawing of nigellidine-4-*O*-sulfite (**4**).

(Figure 1) that confirm the structure of the unique sulfated indazole-type alkaloid unambiguously.

The IR spectrum of **4** displayed intense characteristic absorption bands for sulfur−oxygen bonds at 1234, 1258, and 1272 cm^{-1}. The molecular formula of $C_{18}H_{18}N_2O_5S$ was deduced from the HRESIMS (found 375.1011 $[M + H]^+$, calcd 375.1015 for $[C_{18}H_{18}N_2O_5S + H]^+$). The NMR data of nigellidine (**2**) have still not been reported, though the NMR data of its synthetically prepared methiodide (**2a**) were published by Atta-ur-Rahman et al.[5] The ^1H NMR data of **4**, although recorded in different solvent, are similar to those of the methiodide of nigellidine (**2a**), whereas their ^{13}C NMR spectra differ significantly. The ^{13}C NMR resonances of C-2 (δ_C 161.5), C-3 (δ_C 145.1), C-5 (δ_C 106.9), and C-7 (δ_C 102.3) were tentatively assigned for the methiodide of nigellidine (**2a**), while those for C-14 and C-17 could not be assigned.[5] We unambiguously confirmed the ^{13}C NMR resonances (Table 1) through HMBC correlations (Figure 2) for nigellidine-4-*O*-sulfite (**4**) as δ_C 142.3 (C-2), 111.0 (C-3), 104.2 (C-5), 115.0 (C-7), 115.5 (C-14), and 160.3 (C-17). Remarkable differences of ^{13}C NMR resonances at C-2 ($\Delta\delta_C$ 19.2), C-3 ($\Delta\delta_C$ 34.1), and C-7 ($\Delta\delta_C$ 12.7) were observed. We could also assign the C-14 (δ_C 115.5) and C-17 (δ_C 160.3) resonances unambiguously. The ^1H and ^{13}C NMR assignments were accomplished by analyzing $^1H-^1H$ COSY, ROESY, HSQC, and HMBC spectra.

Compound **4** belongs to a rare class of alkaloids containing the indazole nucleus with a unique sulfate functionality and presumably represents the true natural product leading to nigellidine (**2**) via facile hydrolysis of the sulfate during the isolation process.[5]

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Table 1. NMR Data of Nigellidine-4-*O*-sulfite (**4**)[a] in Comparison with Those of the Methiodide of Nigellidine (**2a**)[b]

no.	δ_C of **4**[a] mult.	δ_C of **2a**[b] mult.	δ_H of **4**[a] mult. (*J*, Hz)	δ_H of **2a**[b] mult. (*J*, Hz)
2	142.3 s	161.5 s		
3	111.0 s	145.1 s		
4	149.1 s	156.1 s		
5	104.2 d	106.9 d	7.39 s	6.70 s
6	145.7 s	144.1 s		
7	115.0 d	102.3 d	7.40 s	7.14 s
8	142.3 s	148.8 s		
10	47.3 t	49.9 t	4.56 t[d] (5.8)	4.45 m
11	20.4 t	21.3 t	2.15 m	2.30 m
12	19.7 t	20.7 t	2.24 m	2.20 m
13	49.2 t	48.0 t	4.46 t[d] (6.0)	4.57 m
14	115.5 s	–[c]		
15	133.4 d	133.3 d	7.61 d (8.8)	7.41 br d (8.8)
16	115.9 d	116.5 d	6.98 d (8.8)	6.90 br d (8.8)
17	160.3 s	–[c]		
18	115.9 d	116.5 d	6.98 d (8.8)	6.90 br d (8.8)
19	133.4 d	133.3 d	7.61 d (8.8)	7.41 br d (8.8)
20	23.1 q	23.0 q	2.53 s	2.57 s
O*Me*		56.3 q		3.83 s

[a] Recorded in DMSO-d_6. [b] Reported in $CDCl_3$ by Atta-ur-Rahman et al.[5] [c] Not observed by Atta-ur-Rahman et al. [d] Triplet-like.

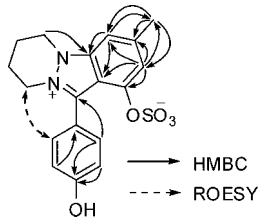

Figure 2. HMBC and ROESY correlations of **4**.

A single-crystal X-ray diffraction study was conducted for nigellidine-4-*O*-sulfite (**4**). A single crystal, of approximate dimensions 0.10 × 0.25 × 0.27 mm, was used for data collection on a Bruker Smart Apex II system,[10] using Cu Kα radiation with a graphite monochromator, fine-focus sealed tube. The crystal was kept at 100 K under a stream of cooled nitrogen gas from a KRYOFLEX low-temperature device. Nigellidine-4-*O*-sulfite (**4**) crystallizes from MeOH in the triclinic space group P_1, with one molecule in the asymmetric unit and two molecules per unit cell ($Z = 2$). The cell dimensions are $a = 8.6602(4)$ Å, $b = 10.0129(4)$ Å, $c = 10.7964(4)$ Å, $\alpha = 73.781(2)°$, $\beta = 68.704(3)°$, $\gamma = 72.799(2)°$, $V = 817.57(6)$ Å^3. Data collection, indexing, and initial cell refinements were all carried out using APEX II software. Frame integration and final cell refinements were done using SAINT software.[11] The final cell parameters were determined from least-squares refinement on 2792 reflections, with $R = 0.020$ and $wR(F^2) = 0.091$. Structure solution, refinement, graphics, and generation of publication materials were performed using SHELXTL, V6.12 software.[12] Hydrogen atoms were placed at their expected chemical positions using the HFIX command and were included in the final cycles of least-squares with isotropic U_{ij}'s related to the atoms ridden upon. The supplementary crystallographic data can be obtained free of charge from The Cambridge Crystallographic Data Centre, reference number CCDC 678552, via www.ccdc.cam.ac.uk/data_request/cif.

Experimental Section

General Experimental Procedures. The UV spectrum was obtained on a Hewlett-Packard 8453 UV/vis spectrometer. IR spectra were recorded on a Bruker Tensor 27 FT-IR spectrometer. The NMR spectra were recorded on a Varian AS 400 NMR spectrometer in DMSO-d_6. HRESIMS data were obtained on an Agilent Series 1100 SL mass spectrometer. Column chromatography was performed using silica gel (J. T. Baker, 40 μm for flash chromatography). TLC was carried out on silica gel 60 F_{254} plates (Merck, Germany).

Plant Material. The seeds of *Nigella sativa* were purchased from Frontier Natural Products CO.OP and identified by Dr. V. Joshi, plant taxonomist at the National Center for Natural Products Research, University of Mississippi, Oxford, MS, where a voucher specimen (2924-NISAR) has been deposited.

Extraction and Isolation. Following removal of the hexanes-soluble part (310 g), the methanolic extract (362 g) of black cumin (1.65 kg) was subjected to column chromatography (CC) over reversed-phase silica (Rp-18) [H_2O−MeOH {1:0 (2 L), 4:1 (1 L), 3:1 (1 L), 7:3 (1 L), 6.5:3.5 (1 L), 3:2 (1 L), 5.5:4.5 (1 L), 1:1 (1 L), 3:7 (1 L), 1:3 (1 L), 1:4 (1 L), 0:1 (1 L)}]. The fractions eluted with 80%−20% H_2O were combined and resolved into 10 fractions by CC over silica gel [$CHCl_3$−MeOH−H_2O (65:35:10, lower layer)]. Purple needles of nigellidine-4-*O*-sulfite (**4**, 10 mg) were obtained from fraction 4 by crystallization from MeOH.

Nigellidine-4-*O*-sulfite (4): purple needles; UV (CH_3OH) λ_{max} nm (log ϵ) 330.7 (4.728), 282.5 (4.725); IR ν_{max}^{KBr} 3238, 1611, 1587, 1449, 1372, 1272, 1258, 1234 cm^{-1}; ^{1}H and ^{13}C NMR (DMSO-d_6), see Table 1; positive HRESIMS *m/z* found 375.1011 $[M + H]^+$ (calcd 375.1015 for $[C_{18}H_{18}N_2O_5S + H]^+$), found 397.0814 $[M + Na]^+$ (calcd 397.0834 for $[C_{18}H_{18}N_2O_5S + Na]^+$), found 413.0547 $[M + K]^+$ (calcd 413.0573 for $[C_{18}H_{18}N_2O_5S + K]^+$).

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Supporting Information Available: ^{1}H and ^{13}C NMR, COSY, HSQC, HMBC, ROESY, IR, and HRESIMS spectra, and CIF file of X-ray crystallographic data of nigellidine-4-*O*-sulfite (**4**). This material is available free of charge via the Internet at http://pubs.acs.org.

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