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Unusual intramolecular *N*→*O* acyl group migration occurring during conjugation of (−)-DHMEQ with cysteine

Ikuko Kozawa [a], Kuniki Kato [a], Toshiaki Teruya [b], Kiyotake Suenaga [b], Kazuo Umezawa [a,*]

[a] Department of Applied Chemistry, Faculty of Science and Technology, Keio University, 3-14-1 Hiyoshi, Kohoku-ku, Yokohama 223-8522, Japan
[b] Department of Chemistry, Faculty of Science and Technology, Keio University, 3-14-1 Hiyoshi, Kohoku-ku, Yokohama 223-8522, Japan

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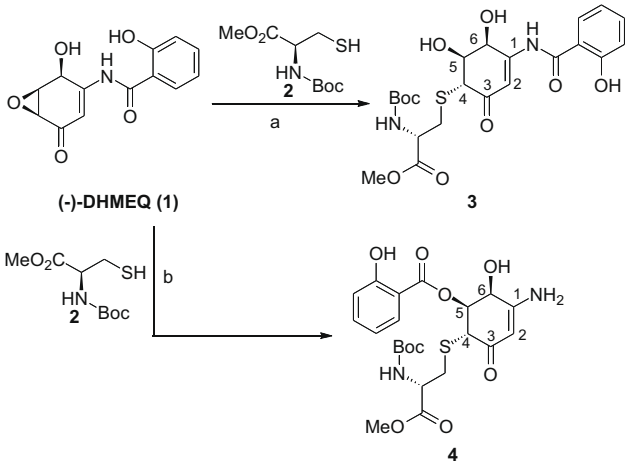
ABSTRACT

Previously we found that (−)-DHMEQ, a specific NF-κB inhibitor, covalently bound to a specific cysteine of NF-κB component proteins. In the course of formation of the (−)-DHMEQ and protected cysteine conjugate, we observed an unusual intramolecular *N*→*O* acyl group migration.

The inducible transcription factor nuclear factor-kappa B (NF-κB) plays an important role in the regulation of immune, inflammatory, and carcinogenic responses.[1] Whereas the normal activation of NF-κB is necessary for cell survival and immunity, deregulated NF-κB expression is a characteristic phenomenon in inflammatory diseases and cancer development.[2] Hence, NF-κB has become a major target in drug discovery, and several natural and synthetic compounds have been investigated for their potential to inhibit NF-κB.[3] Sesquiterpene lactones are the most widely published class of natural products cited as NF-κB inhibitors, and their bioactivity can be explained by a Michael-type conjugate addition of the nucleophilic cysteine sulfhydryl groups of Cys^{38} and Cys^{120} in the p65 monomer of NF-κB to one or more αβ-unsaturated carbonyl moieties of the inhibitor.[4] Recently we designed and synthesized (−)-dehydroxymethylepoxyquinomicin ((−)-DHMEQ, **1**) as a potent and specific inhibitor of NF-κB.[5] Animal and cellular studies have shown **1** to be a promising drug as an anticancer and/or anti-inflammatory agent.[6] Very recently, we clarified that the bioactivity of **1** could be explained by the covalent binding of the nucleophilic cysteine sulfhydryl group of Cys^{38} of p65 to an oxirane ring of **1**, as judged from the data obtained by MALDI-TOF MS analysis.[7] Inter alia we were intrigued by the structure of the conjugate compound and the mechanism of oxirane ring opening by the sulfhydryl group. In this Letter, we report a regioselective oxirane ring opening and an unusual intramolecular N→*O* acyl group migration reaction observed in the course of the formation of the DHMEQ–cysteine conjugate.

When DHMEQ (**1**) was treated with *N*-(*tert*-butoxycarbonyl)-L-cysteine methyl ester (Boc-Cys-Me **2**) in DMF/0.1 M sodium phosphate buffer (1:1, pH 6.0) at room temperature, compound **3**[11] was obtained in 87% yield with complete consumption of **1** within 25 min (Scheme 1).[8]



Scheme 1. Reagents and conditions: (a) compound **2**, DMF/0.1 M sodium phosphate buffer (pH 6.0) (1:1), rt, 25 min; (b) **2**, DMF/0.1 M sodium phosphate buffer (pH 7.0) (1:1), rt, 4 h.

* Corresponding author.
E-mail address: umezawa@applc.keio.ac.jp (K. Umezawa).

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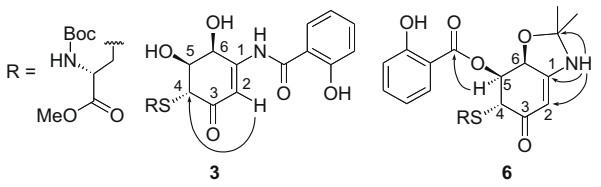
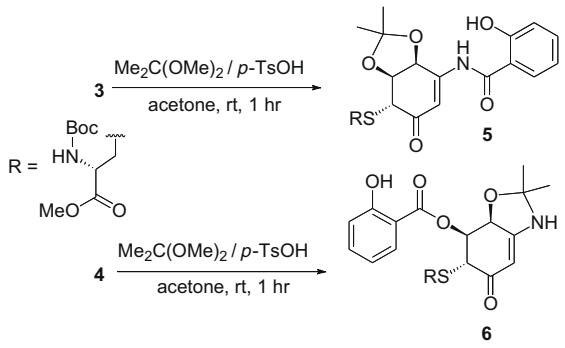


Figure 1. Long-range C–H correlations observed for **3** and **6**.



Scheme 2. Synthesis of acetonides **5** and **6**.

Confirmation of the structure of **3** was provided by MS and 2D NMR spectral analysis using ^{1}H–^{1}H COSY, HMQC, and HMBC. Protons H-5, H-4 and carbon C4 resonances appeared at δ 4.12–4.17 ppm, δ 3.38 ppm, and δ 51.6 ppm, respectively; and a long-range C–H correlation between H-2 and C4 was observed in the HMBC spectrum (Fig. 1).

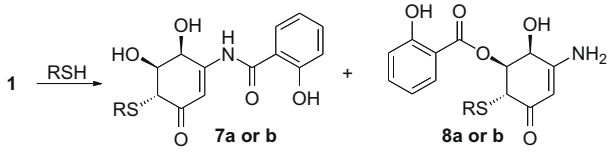
Furthermore, formation of *O*-,*O*-isopropylidene derivative **5** from **3** (Scheme 2) confirmed the regio- and stereochemistry between the two hydroxy groups at C5 and C6 to be in a *cis*-vicinal relationship.

A mechanistic rationalization for the regioselective opening at C4 of the oxirane ring by the sulfhydryl group of **2** is given in Figure 2.[9]

To our surprise, we noticed that the amount of compound **3** decreased with time and therewith a new spot on TLC appeared under the same reaction conditions. After completion of the reaction in 4 h, the unexpected new compound was obtained in 60% yield. NMR studies of compound **4**[12] revealed that 2 characteristic protons, that is, OH at C5 at δ 5.74 ppm and NH of NHC=O at δ 11.82 ppm in **3** disappeared and that resonances of protons H-2 and H-5 appeared at δ 4.99 ppm and δ 5.51 ppm, respectively. However, the structure of **4** presumed by 2D NMR analysis was still open to question because of the absence of the desirable long-range C–H correlation between the carbon of the C=O group at δ 168.7 ppm and H-5 at δ 5.51 ppm. So we prepared the *N*-,*O*-isopropylidene derivative **6**[13] from **4** in order to determine the structures unambiguously (Scheme 2). NMR studies of this product revealed that the proton on NH at δ 9.39 ppm showed a long-range C–H correlation to the isopropyl quaternary carbon at δ 97.2 ppm and that proton H-5 at δ 5.75 ppm showed a long-range C–H correlation to the carbon of the C=O group at δ 167.2 ppm (Fig. 1). Finally, we secured positive evidence for the correct structure of **4**, which was derived from **3** via an unusual intramolecular *N*→*O* acyl group migration reaction in accordance with the hypothetical mechanism depicted in Figure 3.[10]

This unusual oxirane ring opening of DHMEQ with other sulfhydryl compounds followed by this *N*→*O* acyl migration reaction was recognized. Reaction of **1** with mercaptoethanol or benzyl mercaptan in DMF/0.1 M sodium phosphate buffer (pH 7.0) (1:1) for 4 h at room temperature afforded compounds **7a** and **8a** in a 1:7 ratio in 88% yield or compounds **7b** and **8b** in a 1:1 ratio in 87% yield, respectively (Scheme 3).

We previously identified the p65 peptide-(−)-DHMEQ adduct with the dehydrated DHMEQ structure.[7] In this case the peptide adduct had been treated with an acid TFA, then without acidic treatment the intact DHMEQ structure would be likely to be maintained. Treatment of the model adduct molecule was also shown to be dehydrated by the acidic condition.[7] Compared with **3**, it may be more difficult to dehydrate **4**. From this view point, the intact DHMEQ structure without migration may be more likely to exist in the protein. However, time dependent migration of **3** to **4** in the protein is still possible to occur. Crystallographic analysis of the protein adduct may identify the main structure.



Scheme 3. Reagents and conditions: compound **1**, RSH: *a* = $HOCH_2CH_2SH$, *b* = $C_6H_5CH_2SH$, DMF/0.1 M sodium phosphate buffer (pH 7.0) (1:1), rt, 4 h.

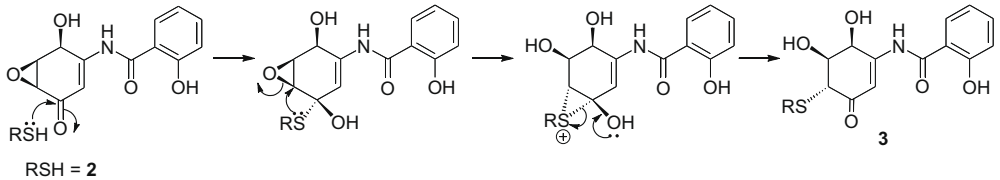


Figure 2. Plausible mechanism of regioselective oxirane ring opening with sulfhydryl group.

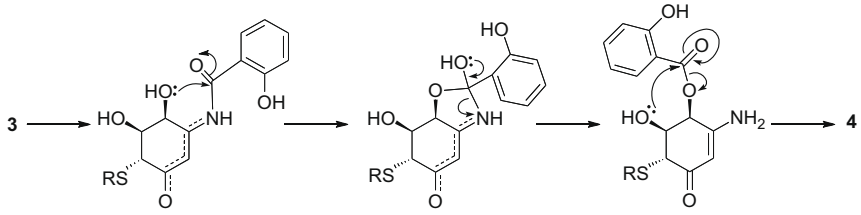


Figure 3. Plausible mechanism of intramolecular *N*→*O* acyl group migration.

In summary, we identified an unusual intramolecular *N*→*O* acyl group migration reaction resulting in compound **4** via compound **3** in the course of the formation of (−)-DHMEQ–cysteine conjugates; however, the detailed reaction mechanism must be the subject of forthcoming investigations. We are intrigued with the speculation that the same migration reaction would happen in the case of the covalent binding of (−)-DHMEQ with NF-κB.

References and notes

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11. For the synthesis of **3** at pH 6.0: To a solution of *N*-(*tert*-butoxycarbonyl)-L-cysteine methyl ester **2** (78 μL, 0.38 mmol) in DMF (8.6 mL) and 0.1 M sodium phosphate buffer (9.6 mL, pH 6.0) was added (−)-DHMEQ (50.0 mg, 0.191 mmol) in DMF (1 mL). The mixture was stirred at rt for 25 min. Then the mixture was diluted with EtOAc (50 mL) and washed with H_2O (30 mL × 2). The organic layer was dried and concentrated in vacuo. The residue was purified by column chromatography on silica gel (EtOAc/hexane, 1:1→5:1) to provide 82.5 mg (87%) of **3** as a colorless oil.: TLC R_f = 0.58 (toluene/EtOH, 3:1); ^{1}H NMR (300 MHz, DMSO-d_6,) δ 1.39 (s, 9H, Boc), 2.97 (m, 2H, cys β), 3.38 (d, 1H, *J* = 2.1 Hz, H-4), 3.65 (s, 3H, COO*Me*), 4.12–4.17 (m, 2H, cys α, H-5), 4.56 (ddd, 1H, *J* = 7.8, 2.1, 2.1 Hz, H-6), 5.74 (d, 1H, *J* = 3.9 Hz, C-5-O*H*), 6.16 (d, *J* = 7.8 Hz, C-6-O*H*), 6.92 (br s, 1H, H-2), 6.96 (d, 1H, *J* = 8.1 Hz, H-5′), 6.99 (dd, *J* = 8.1 Hz, H-4′), 7.41 (d, 1H, *J* = 8.1 Hz, N*H*Boc), 7.44 (ddd, *J* = 8.1, 8.1, 1.7 Hz, H-6′), 7.94 (dd, 1H, *J* = 8.1, 1.7 Hz, H-7′), 11.16 (br s, 1H, C3′-O*H*), 11.82 (br s, 1H, C-1-N*H*); ^{13}C NMR (75 MHz, DMSO-d_6) δ 28.1 × 3, 32.2, 51.6, 52.1, 53.5, 65.7, 72.4, 78.6, 107.2, 117.0 , 118.4, 119.8, 131.2, 134.2, 152.5, 156.2, 156.9, 164.8, 171.3, 193.8; MS (FAB) *m*/*z* 497 $[M+H]^+$.
12. *For the synthesis of* **4**: To a stirred solution of **2** (47 μL, 0.23 mmol) in DMF (4.76 mL) and 0.1 M sodium phosphate buffer (5.76 mL, pH 7.0) was added **1** (30.0 mg, 0.115 mmol) in DMF (1 mL). The mixture was stirred at rt for 4 h. Then the mixture was diluted with EtOAc (30 mL) and washed with H_2O (20 mL × 2). The organic layer was dried and concentrated in vacuo. The residue was purified by column chromatography on silica gel (EtOAc/hexane, 2:1→5:1) to yield 34.4 mg (60%) of **4** as a colorless oil.: TLC R_f = 0.60 (toluene/EtOH, 3:1); ^{1}H NMR (270 MHz, DMSO-d_6,) δ 1.36 (s, 9H, Boc), 3.06 (d, 2H, *J* = 6.6 Hz, cys β), 3.51 (d, 1H, *J* = 3.5 Hz, H-4), 3.64 (s, 3H, COO*Me*), 4.24 (ddd, 1H, *J* = 8.1, 6.6, 6.6 Hz, cys α), 4.76 (m, 1H, H-6), 4.99 (s, 1H, H-2), 5.51 (dd, 1H, *J* = 3.5, 3.5 Hz, H-5), 6.19 (d, *J* = 7.3 Hz, C-6-O*H*), 6.95 (t, *J* = 7.3 Hz, H-6′), 6.99 (d, *J* = 8.0 Hz, H-4′), 7.49 (d, *J* = 8.1 Hz, N*H*Boc), 7.53 (ddd, 1H, 8.0, 8.0, 1.5 Hz, H-5′), 7.64, (dd, 1H, *J* = 8.0 1.5 Hz, H-7′), 10.35 (br s, 1H, C3′-O*H*); ^{13}C NMR (68 MHz, DMSO-d_6) δ 28.1 × 3, 33.0, 49.1, 52.1, 53.9, 63.8, 75.8, 78.5, 93.5, 113.0, 117.4, 119.4, 130.1, 135.9, 155.3,160.1, 164.7, 167.8, 171.4, 187.0; MS (FAB) *m*/*z* 497 $[M+H]^+$.
13. *For the synthesis of* **6**: To a stirred solution of **4** (52.3 mg, 0.105 mmol) in acetone/$Me_2C(OMe)_2$ (2 mL, 1:1) was added a catalytic amount of *p*-TsOH. The mixture was stirred at rt for 1 h. Then the mixture was diluted with EtOAc (20 mL) and washed with H_2O (10 mL × 2). The organic layer was dried and concentrated in vacuo. The residue was purified by column chromatography on slica gel (EtOAc/hexane, 1:1→2:1) to obtain 18.6 mg (33%) of **6** as a colorless oil.: TLC R_f = 0.2 (EtOAc /hexane, 2:1); ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.26 (s, 3H), 1.36 (s, 9H), 1.42 (s, 3H), 3.06 (2d, 2H, *J* = 5.2 Hz), 3.11 (d, 1H, *J* = 2.4 Hz), 3.64 (s, 3H), 4.24 (ddd, 1H, *J* = 8.1, 5.2, 5.2 Hz), 4.85 (s, 1H), 5.32 (d, 1H, *J* = 2.4 Hz), 5.75 (dd, *J* = 2.4, 2.4 Hz), 6.93 (t, *J* = 7.2 Hz), 6.99 (d, *J* = 8.3 Hz), 7.50 (d, *J* = 8.1 Hz), 7.51 (ddd, 1H, *J* = 8.3, 8.3, 1.7 Hz), 7.58, (dd, 1H, *J* = 8.3, 1.7 Hz), 9.39 (s, 1H), 10.28 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 26.4, 27.8, 28.1 × 3, 33.7, 48.4, 52.1, 53.9, 72.0, 74.6, 78.5, 87.2, 97.2, 112.8, 117.6, 119.5, 129.6, 135.9, 155.3, 160.1, 160.3, 167.2, 171.3, 187.5.