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Reactions of Roquefortin with Alkyl Chloroformates

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Abstract–Reactions of roquefortin with alkyl chloroformates provide 3-(1-(alkoxycarbonyl)imidazol-4-yl-methylene]-10b-(1,1-dimethyl-2-propenyl)-5a,10b,11,11a-tetrahydro-2*H*-pyrazinol[1',2':1,5]pyrrolo[2,3-b]-indole-1,4(3H,6H)-diones.

2,5-Diketopiperazine system is a structural core of a number of physiologically active compounds, among them alkaloids produced by microscopic fungi. One of these alkaloids is a neurotoxin roquefortin, 10b-(1,1-dimethyl2-propenyl)-3-(imidazol-4-ylmethylene)-5a,10b,11,11a-tetrahydro-2*H*-pyrazino[1',2':1,5]pyrrolo[2,3-b]indole-1,4(3H,6H)-dione (**I**) [1–3]. An exocyclic double C=C bond at the diketopiperazine ring (in the didehydrohistidine part of molecule **I**) provides a possibility of the presence of two isomers. Compound **I** was formerly shown to possess 3*E*-configuration in contrast to the other microbial alkaloids containing in their structure α,β-didehydroamino acids [4]. Under direct sunlight compound **I** in methanol solution undergoes isomerization into a photoisomer with a *Z*-configuration at the $\Delta^{3(12)}$ double bond [5]. Later on the basis of Overhauser effect in the ^{1}H NMR spectrum of roquefortin supported by carrying out its degradation to L-tryptophan Yamaguchi *et al.* established the absolute configuration of the alkaloid [6]. However some assignments of signals in the ^{1}H NMR spectrum presented in this study are dubious or lacking.

Now the physiological effect of roquefortin (**I**) is extensively studied, but its chemical properties are not sufficiently understood. We investigated the reaction between roquefortin (**I**) and alkyl chloroformates, established the structure of compounds **II** and **III** thus obtained, and refined some assignments of signals in the ^{1}H NMR spectrum of roquefortin (**I**). The reaction of compound **I** with alkyl chloroformates is a fast process yielding a single product. When the reaction was carried out in solution potassium carbonate was added into the reaction mixture for scavenging the liberated hydrogen chloride and facilitating the reaction product isolation. The reaction may be also performed directly on the chromatographic plate; here the only product is butoxycarbonyl derivative **III**. The arising compounds **II** and **III** as also initial roquefortin (**I**) absorb UV light and are identically dyed by Ehrlich reagent.

Table 1. Characteristics of mass spectra of compounds **I**–**III**, *m/z* (I_{rel}, %)

Compd. no.	Molecular ion, M^+	Characteristic fragments in mass spectrum				
		$[M-C_5H_9]^+$	$[M-C_5H_9-R]^+$	$[M-C_5H_9-COO-R]^+$	$C_{10}H_9N_2$	$C_9H_8N^+$ (quinolinium cation)
I	389 (34)	320 (100)	–	–	157 (36)	130 (65)
II	461 (37)	392 (100)	364 (6)	320 (42)	157 (36)	130 (60)
III	489 (45)	420 (100)	364 (8)	320 (68)	157 (32)	130 (57)

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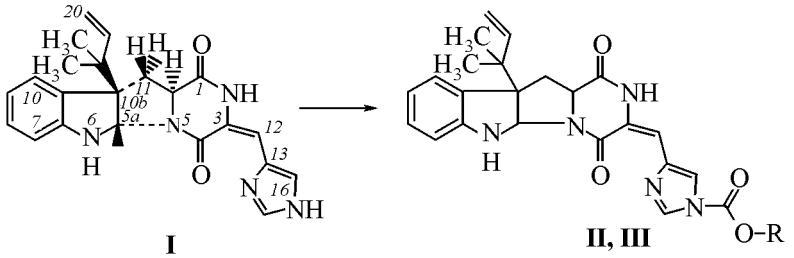

I **II, III**

In the mass spectra of compounds **II** and **III** (Table 1) appear sufficiently intense peaks of molecular ions that easily lose a dimethylallyl radical, then to some extent occurs elimination of the corresponding R radical (β-cleavage with respect to carbonyl group), but the main process involves elimination of the ester group to yield a stable conjugated polycyclic structure.

Although unsubstituted indoline is easily acetylated in good yield, under our reaction conditions no acylation at the nitrogen in the indoline part of roquefortin molecule is observed [7]. The alkoxycarbonyl group replaced hydrogen exclusively at the nitrogen in the imidazole part of the molecule in *16* position as showed data of UV and NMR spectroscopy. In keeping with [8, 9] in the UV spectra of diketopiperazine alkaloids acetylated at the indoline fragment appears a strong absorption band in 246–256 nm range. Yet in the UV spectra of compounds **II** and **III** as well as in that of roquefortin (**I**), its photoisomer, and 3,12-dihydro derivative two absorption bands are present with λ_{max} 312 and 235 nm respectively. The difference in the histidine part of these five molecules weakly affects the common chromophor, and thus their UV spectra are distinguished only by intensity ratio and minor shifts of absorption maxima [10].

Aiming at unambiguous proof of the structure of compound **II** (and of compound **III** by analogy) we studied ^{1}H and ^{13}C NMR spectra (Table 2). The data of different studies on ^{13}C NMR spectra of roquefortin are well consistent [2, 5], yet notable discrepancies exist with respect to assignment of proton signals belonging to benzene and imidazole rings and NH groups [2, 3, 6]. The assignment we suggested for protons H^{15} and H^{17} both in the ^{1}H NMR spectra of roquefortin and compound **II** were confirmed by the value of coupling constants $^1J_{CH}$ measured by the ^{13}C satellites in the ^{1}H NMR spectrum. The assignment of benzene ring protons and NH groups was proved by spectra 2D COSY and deuterium exchange. Among two tautomers of roquefortin the tautomer H^{16} (and not H^{14}) is apparently prevailing since according to X-ray diffraction analysis a compound with a similar imidazole fragment, 9-O-*p*-bromobenzoylmeleagrin monohydrate (metabolite formed from roquefortin by biosynthetic way), exists in this tautomeric form [11].

No signal from NH proton of imidazole ring in the ^{1}H NMR spectrum of compound **II** and considerably different chemical shifts of H^{15} and H^{17} compared to those in the roquefortin spectrum, all the other chemical shifts of CH-protons being similar, show that in compound **II** the NH-proton in the imidazole ring is replaced by ethoxycarbonyl group.

Comparison of ^{13}C NMR spectra of compounds **I** and **II** allows a conclusion that group $COOC_2H_5$ in compound **II** is attached just to N^{16} atom and not to N^{14}. This is evidenced by the upfield shift of C^{17} carbon signal [δ 134.3 in roquefortin (**I**), 120.0 in compound **II**] and downfield shift of that belonging to C^{13} removed from the substituted nitrogen by two bonds [δ 125.5 in roquefortin (**I**), 136.2 in compound **II**]. Analogous changes in chemical shifts were observed by going from the ^{13}C NMR spectrum of imidazole [12] to that of *N*-acetylimidazole [13]. The conclusion on substitution of the hydrogen at N^{16} atom in compound **II** was supported by analysis of $^3J_{CH}$ values. As shown in [13], in the *N*-acetylimidazole these constants are over 10 Hz in coupling through the bond system –N=, and are no greater than 6 Hz in coupling through the >NCOR moiety. In our case $^3J_{(C^{15},H^{17})}$ 6 Hz and $^3J_{(C^{17},H^{15})} < 6$ Hz; consequently, the acyl substituent is linked to N^{16} atom. Thus the reaction of roquefortin (**I**) with alkyl chloroformates affords exclusively compounds **II** and **III**.

The obtained derivatives of ethyl and butyl chloroformates **II** and **III** are sufficiently stable in polar and nonpolar solvents and are easily hydrolyzed on addition of acids and alkali in water solutions. The alkaline hydrolysis is milder and yields the initial compound **I**. At acid hydrolysis deeper degradation of compounds **II** and **III** occurs as is expected since molecule **I** is known to suffer under these conditions

Table 2. Chemical shifts (δ, ppm) and coupling constants (J_{HH}, Hz) [J_{HH}, Hz] in ^{1}H and ^{13}C NMR spectra of compounds **I, II**

Atom no.	^{1}H NMR spectrum		^{13}C NMR spectrum	
	I	**II**	**I**[a]	**II**[b]
1			166.7 s	166.8 s
2	9.19 s[c]	8.39 s[c]		
3			121.9 s	125.8 s
4			159.2 s	157.9 s
5a	5.63 s	5.63 s [165][d]	78.3 d	77.9 d [164.5, 6.0]
6	4.95 s[c]	4.97 s[c]		
6a			149.8 s	148.3 s
7	6.59 d.d (7.5, 1.0)	6.58 d.d (7.4, 1.0)	109.1 d	109.0 d [159.0, 7.5]
8	7.09 t.d (7.6, 1.2)	7.08 t.d (7.6, 1.1)	128.9 d	128.9 d [158.0, 6.0]
9	6.76 t.d (7.5, 1.0)	6.74 t.d (7.5, 1.0)	119.0 d	118.8 d [161.0, 7.0]
10	7.17 d.d (7.5, 1.2)	7.15 d.d (7.5, 1.1)	125.0 d	125.1 d [157.0, 9.5]
10a			128.5 s	128.6 s
10b			61.5 s	61.4 s
11	2.46 d.d (12.4, 11.4)	2.45 d.d (12.4, 11.3)	36.8 t	36.7 t [136.0]
	2.58 d.d (12.4, 6.0)	2.57 d.d (12.4, 6.0)		
11a	4.05 d.d (11.4, 6.0)	4.05 d.d (11.3, 6.0)	58.8 d	58.8 d [140.4]
12	6.28 s d)	6.38 s	110.9 d	115.4 d [150.5]
13			125.5 s	136.2 s
15	7.69 s d) [208] d)	8.14 s d) [217.5] d)	136.4 d	136.5 d [217.5, 6.0]
16	12.94 br			
17	7.26 s d) [190] d)	8.66 s d) [206] d)	134.3 d	120.4 d [205.0, <6]
18			40.9 s	40.8 s
19	5.97 d.d (17.3, 10.8)	5.97 d.d (17.3, 10.8)	143.2 d	143.3 d [152.0]
20	5.10 d.d (17.3, 1.0)	5.08 d.d (17.3, 1.1)	114.5 d.d	114.5 d.d [155.0, 159.0]
	5.13 d.d (10.8, 1.0)	5.11 d.d (10.8, 1.1)		
21	1.02 s	1.01 s	22.0 q	22.4 q [126.0]
22	1.14 s	1.12 s	22.5 q	22.8 q [127.0]
16-R		1.45 t (CH3, 7.1)		4.49 q (CH2, 7.1)
		14.1 q [CH3, 127.5],		64.6 t [CH2, 152.0],
				150.0 s (NCOO)

[a] According to data in [5] and our data with atom numbering used in the present paper.
[b] The indicated multiplicity concerns signal splitting by $^1J_{CH}$.
[c] Broadened signal.
[d] Coupling constant $^1J_{CH}$ was measured from satellites produced by ^{13}C in the ^{1}H NMR spectrum.
[e] Weak coupling of the marked protons was revealed by double resonance and 2D COSY spectra.

a fragmentation yielding as the main product (*E*)-3-(1*H*-imidazol-4-ylmethylene)-6-(1*H*-indol-3-ylmethyl)-2,5-piperazinedione [4].

EXPERIMENTAL

NMR spectra were registered on spectrometer Varian UNITY+400 at operating frequencies 400 (^{1}H) and 100.2 (^{13}C) from solutions in $CDCl_3$. As internal reference were used residual proton signals of the solvent (δ_H 7.24) and its carbon signals (δ_C 76.9). Electron absorption spectra were recorded on spectrophotometer Shimadzu UV-160A. TLC was performed on Silufol UV-254 plates, eluent chloroform–methanol-25% aqueous ammonia, 90 : 10 : 0.1; development under UV irradiation or with Ehrlich reagent. Elemental composition of compounds obtained and their main fragments were determined from the high-resolution mass spectra measured on Finnigan MAT 8430 instrument at ionizing electrons energy 70 eV.

3-[1-(Ethoxycarbonyl)imidazol-4-ylmethylene]-10b-(1,1-dimethyl-2-propenyl)-5a,10b,11,11a-tetrahydro-2*H*-pyrazino[1′,2′:1,5]pyrrolo[2,3-b]indole-1,4-(3*H*,6*H*)-dione (II). To a solution of 0.02 g (0.052 mmol) of alkaloid **I**in 2 ml of chloroform was added potassium carbonate and 20 μl (21.6 mg, 0.2 mmol) of ethyl chloroformate. The mixture was stirred at room temperature for 10 min. The precipitate was filtered off, washed with chloroform on the filter. The filtrate was evaporated at reduced pressure, and the residue was subjected to chromatography. Compound **II**, R_f 0.70, was eluted from the plate with methanol. Yield 0.019 g (80%), mp 207–209°C (from methanol). UV spectrum (methanol), λ_{max}, nm (lgχ): 206.6 (4.50), 235.6 (4.18), 312.4 (4.30). Found M^+ 461.2060. $C_{25}H_{27}N_5O_4$. Calculated *M* 461.2063.

3-[1-(Butoxycarbonyl)imidazol-4-ylmethylene]-10b-(1,1-dimethyl-2-propenyl)-5a,10b,11,11a-tetrahydro-2*H*-pyrazino[1′,2′:1,5]pyrrolo[2,3-b]indole-1,4-(3*H*,6*H*)-dione (III). The reaction was carried out directly on the chromatographic plate. The solution of 2 mg of alkaloid **I** in chloroform was applied as a band on the chromatographic plate, the plate was dried at room temperature, and into the same zone was applied the butyl chloroformate (5 μl in 50 μl of chloroform). The plate was kept for 10 min and then subjected to chromatography. Compound **III**, R_f 0.75, was eluted with methanol, the solution was evaporated. UV spectrum (methanol) λ_{max}, nm: 205.6, 235.0, 312.2. Found M^+ 489.2371. $C_{27}H_{31}N_5O_4$. Calculated *M* 489.2376.

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