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A new decomposition product of dihydroartemisinin

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After prolonged storage, samples of dihydroartemisinin were found to contain a decomposition product with a molecular weight of 238, as evidenced by MS analysis, the formation of which was accelerated by heating at 60 °C for several days. This decomposition product was isolated and identified as 2-(3-oxobutyl)-3-methyl-6-(2-propanal)-cyclohexanon, known to be formed by thermolysis of dihydroartemisinin at 190 °C. However, during work-up of this compound a hitherto unknown decomposition product of dihydroartemisinin with a molecular weight of 210 was obtained and identified by ^{1}H, ^{13}C and two-dimensional NMR spectroscopy as 2-(3-oxobutyl)-3-methyl-6-ethyl-cyclohexanon. Most probably this product was formed by oxidation of the aldehyde functionality of the former decomposition product and subsequent decarboxylation.

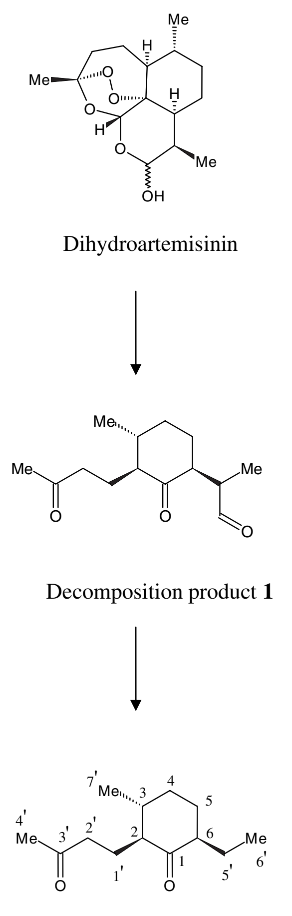
1. Introduction

Artemisinin is a natural product with antimalarial properties that originates from the plant *Artemisia annua*. It can chemically be reduced to dihydroartemisinin, which was reported to be more active than the parent compound, and which serves as a precursor for the antimalarial drugs artesunate and artemether (Meshnick et al. 1996; Sriram et al. 2004). Because dihydroartemisinin is being investigated as a new antimalarial drug, it is important to identify all decomposition products (Wilairatana et al. 2002). Prolonged storage of dihydroartemisinin resulted in the formation of a decomposition product with a molecular weight of 238, as evidenced by MS analysis. The aim of our work presented here was to isolate and to identify this decomposition product, and resulted in addition in the characterisation of a new degradation product of dihydroartemisinin.

2. Investigations, results and discussion

TLC analysis of the parent dihydroartemisinin sample yielded a major spot attributed to dihydroartemisinin (R_f 0.58) and a minor spot due to decomposition product **1** (Scheme) (R_f 0.65). After preparative TLC, the putative decomposition product was isolated. However, when the isolate was analysed by TLC, a different spot with R_f 0.73 was obtained (decomposition product **2**). It was believed to be formed by a degradation reaction of product **1**. To verify this hypothesis, the stock solution of the dihydroartemisinin sample was evaporated to dryness under reduced pressure and dissolved again. After repeating this action several times, the solution was again analysed on TLC. The major spot from dihydroartemisinin remained, but the minor spot, originally due to decomposition product **1**, was shifted to a higher R_f, attributed to degradation product **2**. To prevent degradation, the isolation by preparative TLC was repeated but the compound was dried under

Scheme



Dihydroartemisinin

Decomposition product **1**

Decomposition product **2**

Table: ^{1}H and ^{13}C NMR assignments of 2-(3-oxobutyl)-3-methyl-6-ethyl-cyclohexanon (decomposition product 2) ($CDCl_3$, 400 MHz)

C no.	^{13}C NMR δ (ppm)	^{1}H NMR δ (ppm), mult., J (Hz)
1	213.58	–
2	56.63	1.95, m
3	40.40	1.45, m
4	34.77	1.76, m/1.42, m
5	33.26	2.05, m/1.20, m
6	52.45	2.08, m
1′	20.24	1.70, m (2 H)
2′	41.44	2.49, m/2.30, m
3′	209.20	–
4′	29.83	2.05, s
5′	22.11	1.68, m/1.10, m
6′	11.73	0.81, t, 7.4 Hz
7′	20.62	1.01, d, 6.0 Hz

nitrogen. The isolate was analysed on TLC and the expected spot of decomposition product **1** (R_f 0.65) was observed.

Decomposition product **1**, with a molecular weight of 238 (m/z 261 in ESI MS, $[M + Na]^+$) which was detected in dihydroartemisinin samples, was identical to 2-(3-oxobutyl)-3-methyl-6-(2-propanal)-cyclohexanon, known as a thermolysis product of dihydroartemisinin (Lin et al. 1986). Decomposition product **2** was identified as 2-(3-oxobutyl)-3-methyl-6-ethyl-cyclohexanon, with a molecular weight of 210 (m/z 211 in ESI MS, $[M + H]^+$). The latter compound is a new degradation product of dihydroartemisinin. Its structure was elucidated by NMR spectroscopy. The ^{13}C NMR spectrum displayed two carbonyl groups (δ 213.58 and δ 209.20). Apart from these, only signals in the aliphatic region of the spectral range were observed: three CH-groups (δ 56.63, δ 52.45 and δ 40.40), five CH_2-groups (δ 41.44, δ 34.77, δ 33.26, δ 22.11 and δ 20.24), and three CH_3-groups (δ 29.83, δ 20.62 and δ 11.73). The ^{1}H NMR spectrum displayed a very complex pattern between 0.7 and 2.6 ppm. Based on the HSQC correlations, a singlet at δ 2.05, a doublet at δ 1.01 (J = 6.0 Hz), and a triplet at δ 11.73 (J = 7.4 Hz) could be attributed to the three methyl groups, and also all other correlations between ^{1}H and ^{13}C NMR signals were established. Careful analysis of the two-dimensional NMR spectra allowed to identify decomposition product **2** as 2-(3-oxobutyl)-3-methyl-6-ethyl-cyclohexanon. Its complete ^{1}H and ^{13}C NMR assignments are listed in the Table. Obviously degradation product **2** can be formed by oxidation and subsequent decarboxylation of its putative precursor 2-(3-oxobutyl)-3-methyl-6-(2-propanal)-cyclohexanon, which is known as a decomposition product formed by thermolysis of dihydroartemisinin at 190 °C. The latter compound was a mixture of two diastereoisomers based on the mechanism by which it is formed (Lin et al. 1986). The two diastereoisomers had a different configuration at C-6. Therefore, two sets of ^{13}C NMR signals were observed, which were listed but not assigned, attributed to the major and the minor isomer. However, decomposition product **2** only produced one set of NMR signals, indicating that it was not a mixture of diastereoisomers. Its relative configuration as displayed in the Scheme was based on the configuration of the major diastereoisomer, which is in fact identical to the configuration of artemisinin and dihydroartemisinin (Lin et al. 1986). The reason why decomposition product **2** was not isolated as a mixture of diastereoisomers is not known. Decomposition product **2** has not yet been described as a degradation or thermolysis product of dihydroartemisinin.

Also the thermal rearrangement and decomposition of artemisinin has been investigated; however, its decomposition products were different from those described for dihydroartemisinin (Christen and Veuthey 2001; Lin et al. 1985).

3. Experimental

3.1. General

A Bruker 3000 plus ion-trap mass spectrometer with an electrospray ion source was used to analyse the mass of the compounds. The samples were introduced in the MS by direct injection via a syringe pump. Measurements were performed in positive ion mode.

NMR-spectra were obtained using a Bruker DRX-400 instrument, operating on 400 MHz for ^{1}H and 100 MHz for ^{13}C and $CDCl_3$ was used as solvent. ^{1}H, ^{13}C (including DEPT-135 and DEPT-90) and two-dimensional NMR spectra were taken on a multinuclear broadband inverse probe, including gradient-enhanced COSY (^{1}H, ^{1}H correlations), HSQC (one-bond C−H correlations) and HMBC (long-range C−H correlations).

TLC was carried out on silica gel 60 F_{254} plates (Merck). For preparative TLC, plates with a layer thickness of 0.5 mm (Merck) were used. Solvents were $CHCl_3$ and MeOH from Acros Organics and toluene from Fisher Chemicals.

3.2. Dihydroartemisinin samples

Dihydroartemisinin samples were supplied by Dafra Pharma, Turnhout, Belgium. A stock solution of the dihydroartemisinin sample was prepared in toluene.

3.3. Isolation

The products were isolated by preparative TLC, using $CHCl_3$/MeOH (95 : 5) as the mobile phase. For analysis by TLC the spots were visualised using a spraying reagent consisting of 0.5 g vanillin in concentrated H_2SO_4 (80 ml) and EtOH (20 ml).

2-(3-oxobutyl)-3-methyl-6-(2-propanal)-cyclohexanon (**1**); ESI MS m/z: 261 $[M + Na]^+$.

2-(3-oxobutyl)-3-methyl-6-ethyl-cyclohexanon (**2**); ESI MS m/z: 211 $[M + H]^+$; ^{1}H NMR ($CDCl_3$, 400 MHz); ^{13}C NMR ($CDCl_3$, 100 MHz): see Table.

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