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THERMAL DECOMPOSITION PRODUCTS OF DIHYDROARTEMISININ (DIHYDROQINGHAOSU)

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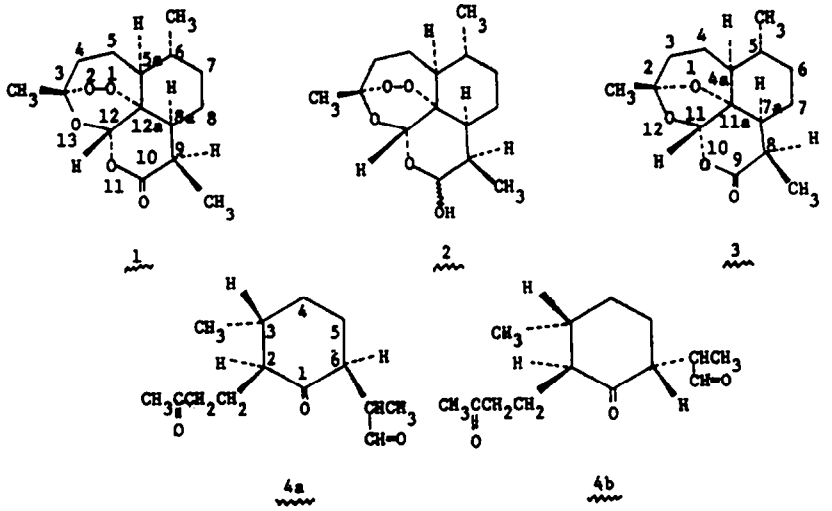
Abstract- Dihydroartemisinin (2), a sodium borohydride reduction product of artemisinin (1), undergoes thermolysis at 190 °C to give desoxyartemisinin (3) and a preponderant decomposition product (4) consisting of 2 epimers 4a, (2S, 3R, 6S)-2-(3-oxobutyl)-3-methyl-6-[(R)2-propanal]-cyclohexanone, and 4b, (2S, 3R, 6R)-2-(3-oxobutyl)-3-methyl-6-[(R)2-propanal]-cyclohexanone.

Artemisinin (qinghaosu, 1), a clinically useful antimalarial agent isolated from the plant Artemisia annua, is an unusual sesquiterpene lactone which contains an epidioxide function.[1-7] Dihydroartemisinin (2), obtained by the sodium borohydride reduction of 1, was reported[8] to be more therapeutically active than the parent compound. Early studies in this laboratory demonstrated that 1 rearranges and decomposes to several products upon heating to 190 °C.[9] Three of these products were identified, none of which is preponderant. In contrast, we have noted that a major thermal decomposition product is formed by heating 2 at 190 °C for 3 minutes. In this communication, we describe the isolation and chemical identification of both the major and minor thermolysis products of 2.

RESULTS AND DISCUSSION

Dihydroartemisinin (2), prepared by sodium borohydride reduction of 1,[1] was heated neat in a round-bottom flask for 3 minutes in an oil bath preheated to 190 °C. Upon cooling to room temperature, the brown mixture was separated by a silica gel column using 15% EtOAc/$CHCl_3$ as eluent to give 3 (30%) and 4 (50%).



Compound 3 crystallized from hexane as colorless leaflets, mp 110-111 °C. CIMS [CH_4, m/z 267 (M+1)] indicates that the molecular weight of 3 is 266. IR shows a 6-membered lactone carbonyl absorption at 1748 cm^{-1} whereas ^1H NMR exhibits a singlet at 5.69 and a multiplet at 3.20 ppm for H-11 and H-8, respectively. Three methyl functions show resonances at 0.94 (br s, 3H), 1.20 (d, J = 7.25 Hz, 3H), and 1.52 ppm (s, 3H) which are compatible in chemical shift to C-6 (0.99 ppm), C-9 (1.12 ppm), and C-3 (1.44 ppm) methyl groups, respectively, of compound 1. ^{13}C NMR spectra indicate that compound 3 consists of 15 carbon atoms and confirmed the existence of a lactone carbonyl

(171.64 ppm). One tertiary carbon (99.54 ppm) attached to two oxygen atoms and two tetra-substituted carbons linked by one and two oxygen atoms (82.31, and 109.07 ppm), respectively, are clearly identifiable. The 11 other carbons are aliphatic in nature (12.53, 18.44, 21.90, 23.42, 23.85, 32.63, 33.39, 33.88, 35.23, 42.33 and 44.55 ppm). These data indicate that compound 3 is the known compound, desoxyartemisinin (lit.,[1] mp 109–110 °C), which has been reported to be a constituent of A. annua,[10] a metabolite of artemisinin in man[11,12] and a catalytic hydrogenation product of artemisinin.[1]

Compound 4 is an oil whose ^{13}C NMR and GC/CIMS (isobutane) spectra indicate that it is a mixture of two isomers (4a and 4b, m.w. 238) in a ratio estimated to be 2.5 : 1 by capillary GC/MS and ^{13}C NMR. Separation by TLC could not be achieved in a variety of solvent systems. However, 4a and 4b could be partially resolved by fused silica capillary column gas chromatography–mass spectrometry. IR (neat) demonstrated a broad carbonyl absorption at 1715 cm^{-1}. ^1H NMR shows the presence of an aldehyde proton at 9.74 ppm, a sharp singlet at 2.13 ppm for methyl function and a multiplet centered around 1.06 ppm for two methyl groups. ^{13}C NMR indicates that both isomers consist of 14 carbons, including one aldehydic, two ketonic and 11 aliphatic with no oxygen attachment. The minor isomer has 6 of 14 carbons with a slight difference in chemical shifts ($\Delta\ \delta \leq 1$ppm) from the major isomer, including the aldehyde and one of the ketone carbonyl carbon atoms.

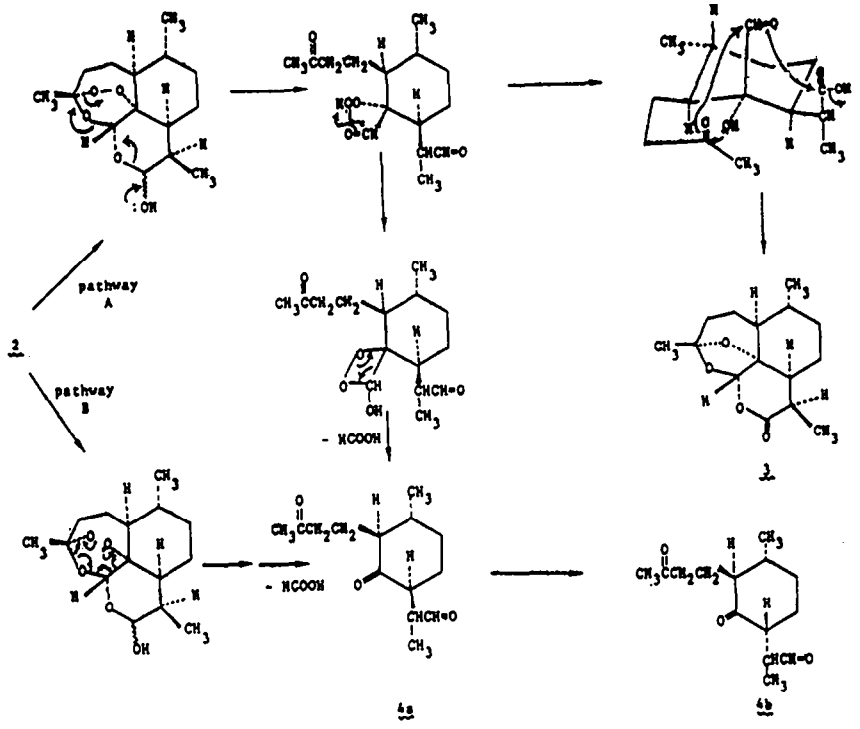
GC/MS data indicate that compound 4 dehydrates readily under chemical ionization mass spectrometric conditions using CH_4 as reagent gas. The base peak is m/z 221 ($M+1 - H_2O$) and the intensity of M+1 peak (m/z 239 ≤ 6% of the base peak) is so weak that it can be easily overlooked. However, a strong M+1 peak (50% of the base peak) was observed when a softer reagent gas, isobutane, was used. The EI fragmentation pattern of 4a and 4b [m/z (% relative intensity) 220 (0.18, $M^+ - H_2O$), 210 (4, $M^+ - CO$), 180 (2.4, M^+ – acetone or propionaldehyde), 152 (15, $M^+ - CO$ – acetone), 123 (4), 69 (17), 55 (31) and 43 (100, $CH_3C{=}O^+$)] are identical but differ from those of CI. The EI spectrum shows no parent peak (m/z 238) and the m/z 220 ($M^+ - H_2O$) is very weak. The most prominent peak is m/z 43 ($CH_3C{=}O^+$) arising from the side chain methyl ketone function of 4.

The spectrometric and CIMS data led to the structure assignment of 4. The observation that the chemical shifts of the aldehyde carbonyl carbon of the 2 isomers differ, whereas the side chain ketone and the C-3 methyl carbons have identical chemical shifts, suggest that the configuration at C-6 and not C-2 position is different in both isomers. Since all 3 bulky groups of 4a are in an equatorial position whereas 4b has a propionaldehyde group occupying an axial position, the major isomer was assigned structure 4a, (2S, 3R, 6S)-2-(3-oxobutyl)-3-methyl-6-[(R)2-propanal]-cyclohexanone, and the minor isomer, 4b, (2S, 3R, 6R)-2-(3-oxobutyl)-3-methyl-6-[(R)2-propanal]-cyclohexanone.

A mechanism that may account for the formation of 3, 4a and 4b is shown in Scheme I. It involves either an ionic (pathway A) or a free radical (pathway B) pathway or both. This is consistent with the observation that the product ratio of 3 to 4 is temperature dependent. Small scale pyrolysis in a NMR tube at 160 °C for 10 minutes give an equal signal intensity to peaks with resonances at 9.74 (aldehyde proton of 4) and 5.69 ppm (H-11 of 3), whereas heating at 190 °C gave a peak ratio of 1:2, in favor of compound 4 formation. Parallel results were observed under GC/MS conditions. Whereas compound 3 is the major (80%) pyrolysis product when a 160 °C injector temperature was used, compound 4 (90%) is preponderant with less than 3% of 3 when the injector temperature was raised to 300 °C. These results suggest that low temperature favors pathway A, whereas high temperature favors the free radical mechanism (pathway B). Based upon the proposed mechanism of formation and product distribution, 4a is probably formed first and then undergoes partial epimerization at C-6 to give a mixture of 4a and 4b.

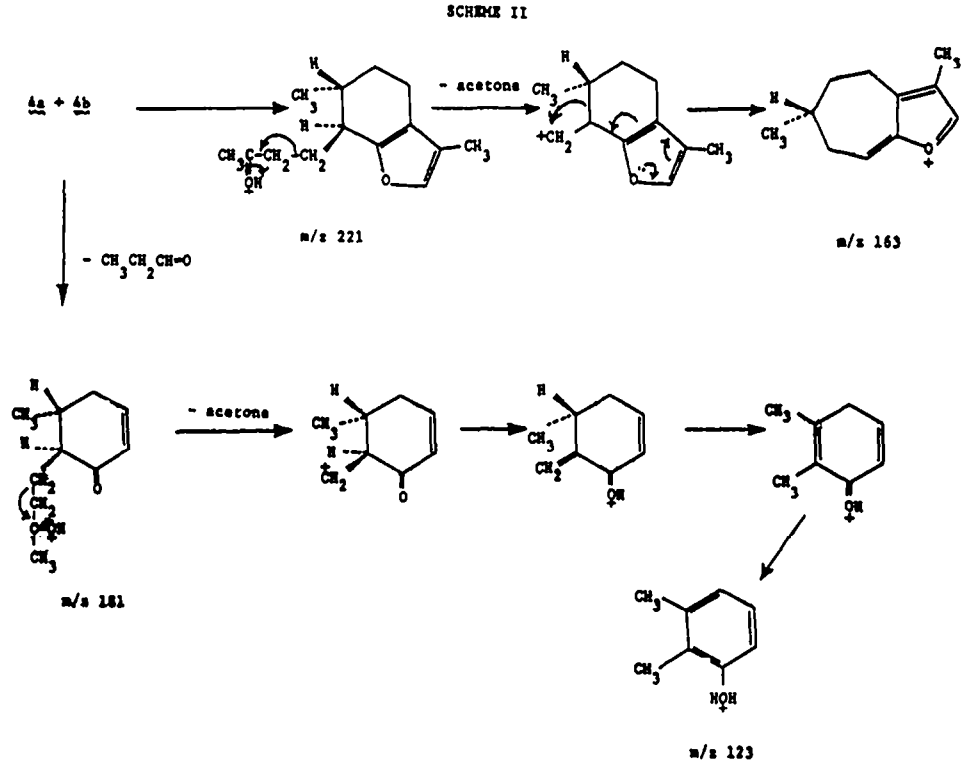
Several factors such as temperature, scale, and length of reaction time appear to effect the yield of the isolated products, 3 and 4. Higher reaction temperatures not only altered the product distribution, but also lowered the total yield of 3 and 4. The intensity of unidentified low R_f brown tlc spots increased when the reaction was carried out in a larger scale or when a prolonged reaction time was used.

SCHEME I



The proposed CIMS fragmentation pattern is shown on Scheme II. Dihydroartemisinin, under GC/CIMS (CH_4) conditions, gives predominantly (i.e., >97%) compound 4 whose base peak is m/z 221. This peak is considered to be diagnostic for dihydroartemisinin and has been used for selective ion GC/MS monitoring of the compound in biological fluids.[13]

SCHEME II



EXPERIMENTAL SECTION

All melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. Infrared spectra of solid samples were obtained in KBr disks on a Perkin-Elmer Model 283 spectrophotometer. NMR spectra were run on a JEOL-90Q spectrometer using Me_4Si as an internal standard and $CDCl_3$ as solvent. Mass spectra were determined on a Nermag R10-10C spectrometer interfaced to an INCOS data system.

Thermal Decomposition of Dihydroartemisinin (2): Dihydroartemisinin[1] (180 mg, 0.63 mmoles) in a round-bottom flask was heated in a silicone oil bath preheated to 190 °C. A vigorous gas evolution took place as soon as the compound melted. The heating was stopped after the gas evolution ceased (2-3 min). The reaction mixture was allowed to cool to room temperature and separation was carried out on a silica gel column using 15% EtOAc/$CHCl_3$ as eluent to give two major products, 3 (50 mg, 30%) and 4 (71 mg, 50%).

Compound 3 was recrystallized from hexane to give colorless leaflets, mp 110 - 111 °C (lit.[1] mp 109-110 °C); CIMS (CH_4): m/z 267 (M+1); IR (KBr): 1748 cm^{-1} (lactone carbonyl); ^1H NMR: δ 0.94 (br s, 3H), 1.20 (d, J = 7.25 Hz, 3H), 1.52 (s, 3H), 3.20 (m, 1H) and 5.69 (s, 1H); ^{13}C NMR: 12.53, 18.44, 21.90, 23.42, 23.85, 32.63, 33.39, 33.88, 35.23, 42.33, 44.55, 82.31, 99.54, 109.07, and 171.64 ppm.

Compound 4 is an oil; IR (neat): 1715 cm^{-1} (carbonyl); ^1H NMR: δ 1.06 (m, 6H), 2.13 (s, 3H) and 9.74 (s, 1H); GC/MS (isobutane) with a fused silica capillary column shows two partially resolved peaks in a ratio of 2.5 : 1. Both peaks gave identical CIMS (CH_4) spectra: m/z (% relative intensity) 239 (6, M+1), 221 (100, M+1 - H_2O), 210 (18, M - CO), 181 (10, M+1 - propionaldehyde), 163 (74, M+1 - H_2O - acetone), 152 (12) and 123 (12, M+1 - propionaldehyde - acetone). Separation of the two isomers could not be achieved by TLC in several solvent systems. ^{13}C NMR spectrum also exhibits two isomers in a ratio of 2.5:1, estimated by the peak height of the corresponding carbons of the two isomers; 4a (major): 10.96, 20.00, 20.44, 29.87, 30.25, 34.42, 40.16, 41.14, 45.25, 51.70, 56.47, 203.63, 208.77 and 211.88 ppm; 4b (minor): 10.96, 20.00, 20.44, 29.43, 29.87, 34.42, 39.30, 41.14, 45.25, 52.40, 56.31, 204.36, 208.77, and 211.05 ppm.

Small Scale Pyrolysis Studies of Dihydroartemisinin: In two NMR tubes containing 10 mg each of dihydroartemisinin were heated separately in a silicone oil bath preheated at 160 °C and 190 °C, respectively, for 10 min. After cooling, ^1H NMR spectra were taken in $CDCl_3$. The peaks at 9.74 (aldehyde proton of 4) and 5.69 ppm (H-11 of 3) were integrated and the ratio of the integrated values was used as the product ratio of 3 and 4.

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