Synthesis of a new series of 10α-nitrodeoxoartemisinin and their antimalarial activity

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Selective replacement of the C-10 hydroxyl group of dihydroartemisinin by a nitro group gives a hitherto unknown derivative of the potent plant based antimalarial drug artemisinin. The nitrostabilized carbanion at C-10 generated by treating 10α-nitrodeoxoartemisinin with variety of bases and subsequent coupling of this carbanion with several electrophiles led to the synthesis of a new series of artemisinin analogues.

Resistance of the malaria parasite to chemotherapeutic agents such as chloroquine has increased to an alarming proportion in the last decade and urgent need for new drugs was highly felt.1-5 Artemisinin 1, a new clinically useful antimalarial agent isolated from Chinese traditional medicinal plant Artemisia annua is an unusual sesquiterpene lactone containing an endoperoxide function. Its unique chemical structure coupled with its low toxicity and proven antimalarial activity has attracted attention from both chemists and pharmacologists since its discovery in China in early 1970s. The practical use of artemisinin as an antimalarial drug was however impaired by (i) its low solubility in water as well as in oil6 (ii) its poor efficacy by oral administration,6 (iii) short plasma half life 8,9 and (iv) the high rate of recrudescence in treated patients.10 Chemical modification of artemisinin has resulted in a number of analogues with improved efficacy and increased solubility in either oil, i.e. artemether 3, and arteether 48 or water, i.e. sodium artesunate 511 and sodium artelinate 6.12 In continuation of our interest on the chemistry of aliphatic nitro compounds and their application for the synthesis of bioactive molecules13 we recently reported14 a convenient method for synthesis of a number of aliphatic nitro compounds directly from alcohols. In order to widen the scope of this reaction, we desired to test this method on more sensitive molecules such as dihydroartemisinin 2, on which we had been working for some time. In this communication we report the synthesis of a new type of artemisinin derivatives which contain nitro function at C-10 and their antimalarial activity.

Results and Discussion

When dihydroartemisinin, 2, prepared by reducing artemisinin 1, with sodium borohydride in methanol at 0 to 5°C as per procedure described in the literature15 was treated with a reagent mixture containing NaNO2, AcOH and HCl at RT without stirring,14 compound 7 was formed in 70% yield as a crystalline solid, m.p.135°C (Scheme 1).

The 'α' relative stereochemistry of the newly introduced nitro group in 7 at C-10 has become evident from the coupling constant (J = 10 Hz) of H-10 which appeared as a doublet at 5.7 ppm in its 1H NMR spectrum. Although we did not comment on the mechanism of this reaction in our earlier communication,14 exclusive formation of the 'α' isomer in this case points towards a SN2 type mechanism for this transformation, where attack of the nucleophilic -NO2 group might be taking place from the less hindered 'α' side of C-10 in 2. Treatment of 10α-nitrodeoxoartemisinin, 7 with a variety of

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1Dedicated to Prof. U.R. Ghatak on his 70th birthday.
electrophiles in presence of a base (see experimental) gave the corresponding addition products via intermediate nitrostabilized carbanion species 'A'. Reaction of 7 with acetaldehyde and amberlyst A-21 resin without solvent gave the addition product 8. Methyl ethyl ketone also reacted in presence of amberlyst A-21 resin giving rise to compound 9 as the sole product. When 7 was reacted with Michael acceptors such as acrolein and acrylonitrile in presence of amberlyst A-21 resin, the corresponding 1,4-addition products 10 and 11 were obtained respectively in good yields.

It is worth mentioning that although we found amberlyst A-21 resin to be the most suitable base for transformation of 7 to 'B' via 'A', other bases like DBU, DBN, KF, etc. also behaved similarly and gave product 'B' in varying yields. It may be noted that although the reactions described for the synthesis of 10α-nitrodeoxoartemisinin 7 could be regarded as straightforward, we observed that on slight change in experimental conditions, the same reagent system when treated with dihydroartemisinin 2, behaved as an oxidant and resulted artemisinin 1 as the major product. The relative stereochemistry of the newly created asymmetric center at C-10 in the compounds 8-11 has been assigned as 'α' on the basis of the conformational analysis with the dreading model of the nitronate anion species 'A', which showed more vacant space on the 'α' face and the 'β' face is relatively more crowded because of the presence of the methyl group with β orientation at C-9. Because of this the approach of the electrophiles towards the carbanion is more favoured from the 'α' face.
Table I—Antiprotozoal screening assays of artemisinin derivatives

<table>
<thead>
<tr>
<th>Sample</th>
<th>P. falciparum</th>
<th>P. falciparum</th>
<th>S.I</th>
<th>IC50 (ng/ml)</th>
<th>IC50 (ng/ml)</th>
<th>S.I</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>(D6 clone)</td>
<td>(W2 clone)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>4.2</td>
<td>79</td>
<td>4.4</td>
<td>75</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>370</td>
<td>&gt;1.3</td>
<td>360</td>
<td>&gt;1.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>280</td>
<td>&gt;1.7</td>
<td>290</td>
<td>&gt;1.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>artemisinin</td>
<td>3.8</td>
<td>&gt;125</td>
<td>3.7</td>
<td>&gt;129</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Sensitivity Index (S.I.) = IC50 (Vero Cells) / IC50 (P. falciparum), NA = Not Active
Retest Run @ 0.2/ml @ 6 concentrations.

Antimalarial activity

The antimalarial activity of the new artemisinin derivatives was evaluated on Plasmodium falciparum (D6 clone) and P. falciparum (W2 clone) by Markler method which involves monitoring parasite LDH enzyme. The evaluation was carried out at 0.2mg/ml concentration and the results are summarized in Table I.

It is evident from Table I that compound 7 has antimalarial activity closer to that of artemisinin, whereas other analogues viz, 9,10 and 11 did not show any appreciable activity against Plasmodium falciparum (D6 clone) and P. falciparum (W2 clone) at this concentration. Although a large number of artemisinin analogues have so far been made and several of them are well known antimalarial drugs available in the market, efforts to make newer analogues with better therapeutic indices are still a matter of high priority. Moreover this is the first report of introduction of a versatile functional group like -NO2 in the sensitive artemisinin molecule without damaging the 1,2,4-trioxane pharmacophore. The nitrostabilized carbanion species 'A' that could be generated by treating 7 with a mild base under ordinary conditions further widens the scope for synthesizing a wide range of artemisinin analogues starting from 10α-nitrodoxono-artemisinin, 7.

Experimental Section

General: Melting points were determined with Buchi 540 melting point apparatus and are uncorrected. Infrared spectra (IR) were recorded on a Perkin-Elmer 236 grating infrared spectrometer. Bruker DPX 300 spectrometer was employed for 1H NMR spectra with TMS as internal reference and shift values are expressed in δ-scale. Mass spectra were recorded at 70 eV in a Finnigan-Matt GC-MS equipment. Silica gel G was used for TLC. The elemental analyses were recorded in a Perkin-Elmer series II CSNS/O Model 2400 analyzer.

Preparation of 7. To a solution of 2 (1.5 gm; 5.3 mmole) in CH2Cl2 (35mL) was added NaN02 (4 g, 57.97 mmole) and acetic acid (5 mL); and left undisturbed at RT for 15 min. Then HCl (6 drops) was added and kept it for 24 h at RT. When TLC observation showed disappearance of the starting material, the reaction mixture was washed with water (3×10 mL). The organic layer was dried over anhyd. Na2SO4 and distilled off to get a crude product which was further, purified by preparative TLC (1:3 EA/PE) to get 7 (1.155 gm; 3.70 mmole, 70%) as a white crystalline solid, m.p. 135-36°C; IR (CHCl3): 1540 (NO2), 1435, 1375, 1300, 1225 and 1130 cm-1; 1HNMR (CDCl3): δ 0.90 (d, J=7.5 Hz, 3H,CH3 at C-11), 0.96 (d, J=6 Hz, 3H,CH3 at C-10), 1.44 (s, 3H,CH3 at C-4), 1.12-2.23 (m, 10H), 2.80 (m, 1H), 3.1 (m, 1H, H-11), 5.70 (d, J=10 Hz, 1H, H-12), 5.90 (s, 1H, H-5); MS: m/z 314 (M+1), 298, 286, etc. Anal. Calcd. for C17H23N06: C, 57.50; H, 7.40; N, 4.47. Found : C, 57.55; H, 7.46; N, 4.50.

Preparation of 8. To a solution of 7 (150 mg, 0.481 mmole) in 15 mL CH2Cl2 was added acetaldehyde (0.4 mL, excess) and 1 g of Amberlyst A-21 and the reaction mixture was stirred at RT for about 14 h (overnight). When TLC showed disappearance of the starting material, the reaction mixture was filtered and the solvent evaporated off to give a crude which was purified by preparative TLC (1:5 EA/PE) to give pure 8 as a white solid (138mg, 0.386 mmole, 80%), m.p.114-15°C; IR (CHCl3): 1340, 2950, 1540, 1435, 1375, 1300, 1225 and 1130 cm-1; 1HNMR (CDCl3): δ 0.90 (d, J=7.5 Hz, 3H,CH3 at C-11), 0.96 (d, J=6 Hz, 3H,CH3 at C-10), 1.44 (s, 3H,CH3 at C-4), 1.12-2.23 (m, 10H), 2.3 (br, 1H, OH), 2.80 (m, 1H), 3.1 (m, 1H, H-11), 3.5 (br, 1H, ), 5.70 (d, J=10 Hz, 1H, H-12), 5.90 (s, 1H, H-5); MS: m/z 358 (M+1), 339, 310, 292, etc. Anal. Calcd. for C17H23NO5: C, 57.13; H, 7.61; N, 3.92. Found : C, 57.18; H, 7.55; N, 3.98.

Preparation of 9. To a solution of 7 (200 mg, 0.641 mmole) in CH2Cl2 (15 mL) was added ethyl methyl ketone (252 mg, 3.0 mL, 3.5 mmole) and Amberlyst A-21 (1 g) and the mixture was stirred at RT for about 15 h. On completion (observed on TLC), the reaction mixture was filtered and the solvent distilled off to give a crude product which on purification by preparative TLC (1:4 EA/PE) gave pure 9 as a solid product. (186 mg, 0.482 mmole, 75%) m.p.111-12°C. IR (CHCl3): 3400, 2950, 1540, 1435, 1375, 1300,1225 and 1130 cm-1; 1HNMR
(CDCl₃): δ 0.90 (d, J=7.5 Hz, 3H, CH₃ at C-11), 0.96 (d, J=6 Hz, 3H, CH₃ at C-10), 1.17 (d, J=7 Hz, CH₃), 1.44 (s, 3H, CH₃ at C-4), 1.36 (d, J=6.8 Hz, 3H, CH₃), 1.12-2.23 (m, 12H), 2.3 (br, 1H, OH), 2.80 (m, IH), 3.1 (m, 1H, H-11), 3.5 (br, 1H), 7.50 (d, J=10 Hz, 1H, H-12), 5.90 (s, 1H, H-5); MS: m/z 386, 367, 338, 320, 305, etc. Anal. Calcd. for C₁₉H₂₇N₂O₇: C, 59.20; H, 8.11; N, 3.67. Found: C, 59.28; H, 8.06; N, 3.60.

Preparation of 10. To a solution of 7 (200 mg, 0.641 mmole) in 10 mL CH₂Cl₂ was added acrolein (0.4 mL, 5.97 mmole) and 1 g of Amberlyst A-21 and the reaction mixture was stirred at RT for about 14 h (overnight). When TLC showed disappearance of the starting material, the reaction mixture was filtered and the solvent evaporated off to give a crude which was purified by preparative TLC (1:5 EA/PE) to give pure 10 as white solid (177 mg, 0.479 mmole, 75%); m.p.118-19°C; IR (CHCl₃): 1740 (CHO), 1450 (N0₂), 1375, 1360, 1250, 1100, and 775 cm⁻¹; ¹HNMR (CDCl₃): δ 0.90 (d, J=7.5 Hz, 3H, CH₃ at C-11), 0.96 (d, J=6 Hz, 3H, CH₃ at C-10), 1.14 (s, 3H, CH₃ at C-4), 1.12-2.23 (m, 12H), 2.1 (m, 2H, -CH₂-CHO), 2.80 (m, 1H), 3.2 (m, 1H, H-11), 5.50 (s, 1H, H-5), 9.4 (t, J=4 Hz, 1H, CHO); MS: m/z 370, 332, 293, 279, etc. Anal. Calcd. for C₁₉H₂₇N₂O₇: C, 58.52; H, 7.37; N, 3.79. Found: C, 58.56; H, 7.39; N, 3.81.

Preparation of 11. To a solution of 7 (100 mg, 0.321 mmole) in 20 mL CH₂Cl₂ was added acrylonitrile (0.3 mL, 24.2 mg, 4.5 mmole) and 1g of Amberlyst A-21 resin and the reaction mixture was stirred at RT for about 14 h (overnight). When TLC showed disappearance of the starting material, the reaction mixture was filtered and the solvent evaporated off to give a crude which was purified by preparative TLC (1:4 EA/PE) to give pure 11 as white solid (81 mg, 0.221 mmole, 68%); m.p. 130-31°C; IR (CHCl₃): 2250(CN), 1540(NO₂), 1435, 1375, 1300, 1225 and 1130 cm⁻¹; ¹HNMR (CDCl₃): δ 0.91 (d, J=7.5 Hz, 3H, CH₃ at C-11), 0.95 (d, J=6 Hz, 3H, CH₃ at C-10), 1.43 (s, 3H, CH₃ at C-4), 1.14-2.24 (m, 12H), 2.11 (m, 2H, -CH₂-CN), 2.75 (m, 1H), 2.95 (m, 1H, H-11), 5.5 (s, 1H, H-5), 2.95 (m, 1H, H-11), 5.5 (s, 1H, H-5), 4.44 (d, J=9.2 Hz, 1H, H-12), 5.34 (s, 1H, H-5); MS: m/z 366, 319, 293, etc. Anal. Calcd. for C₁₅H₂₈N₂O₇: C, 59.00; H, 7.15; N, 7.65. Found: C, 59.06; H, 7.10; N, 7.61.

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References
4. TDR News (News from the WHO Division of Control of Tropical Diseases) 46, 1994, 5.
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