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DIRECTION OF BROMINATION AND NITRATION OF DEOXYPEGANINE AND ITS HYDROCHLORIDE USING HPTLC. SYNTHESIS OF 6-BROMO(NITRO)-, 6,8-DINITRODEOXYVASICINONES, 6-NITRO(BROMO)-, 6,8-DINITRODEOXYPEGANINES, AND 6H(BROMO)PEGANOLS

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*The reaction of deoxypeganine (DOP) (**1**) and its hydrochloride (DOP·HCl) (**2**) with N-bromosuccinimide and a nitrating mixture was studied. It was found that bromination of DOP occurred at C-4 and the aromatic ring. Nitration of DOP·HCl produced either 6-nitro- or 6,8-dinitro-deoxypeganines and 6-nitro-deoxyvasicinone or their mixture in various ratios depending on the substrate:nitrating mixture ratio. 6H- and 6-Br-deoxypeganines were transformed into the 4-hydroxy derivatives, 6H(Br) peganols or deoxyvasicinones. A method for qualitative and quantitative analysis of the pure compounds and the mixture of reaction products using HPTLC was developed.*

Key words: deoxypeganine, deoxyvasicinone, peganol, alkaloids, bromination, nitration, high-performance thin-layer chromatography.

Tricyclic quinazoline alkaloids and their derivatives have been shown to react with electrophilic reagents [1-6]. The direction of the electrophilic substitution reaction at the 6-position of the aromatic ring has been unambiguously proven for quinazol-4-ones, e.g., deoxyvasicinone (DOV, **3**) [1-6]. A second electrophilic substituent could not be introduced into 6-nitrodeoxyvasicinone due to the relatively low nucleophilicity of the aromatic ring resulting from the electronegative substituent in the 6-position, the carbonyl group on C-4, and the presence of the N=C double bond [6]. Bromination of **3** in acetic acid occurs at the α-C atom or in the aromatic ring ($KBrO_3$ + H_2SO_4) [6]. The direction of the reaction changes drastically on going to tricyclic quinazoline alkaloids. Thus, peganine and deoxypeganine react with *N*-bromosuccinimide (NBS) in glacial acetic acid to form the corresponding 6-bromo derivatives [5]. Bromination of DOP by NBS in $CHCl_3$ with subsequent treatment with base gives peganol, 4-hydroxydeoxypeganine (**4**) (20% yield). It was noted that peganol was formed only if "freshly purified" NBS was used [4]. Compound **4** was also synthesized by reaction of DOP with sodium hydride although in very low yield [3]. Compound **4** was formed in high yield by $KMnO_4$ oxidation of DOP hydrochloride in acidic medium [4].

It is well known that peganol is an optically inactive alkaloid that is isolated from the plant *Peganum harmala* [7].

Bromination of DOP by "impure" NBS in $CHCl_3$ gave a mixture of 6-bromodeoxyvasicinone (**10**), 6-bromopeganol (**6**), and 8-bromodeoxyvasicinone [2], with **6** being the main product. Reaction of DOP and NBS in glacial acetic acid with heating on a water bath isolated 6-bromo-DOP hydrobromide (**7**, 12%), 6-bromo-DOV (**10**, 13%), and 6-bromopeganol (**6**, 17%). The ratio of products at room temperature was 85, 2, and 1.3%.

Bromination of peganol by NBS in the presence of benzoyl peroxide in $CHCl_3$ or in glacial acetic acid or by bromine in $CHCl_3$ formed 6-bromopeganol and DOV. The principal product was **6**, although in low yield [2].

Thus, the literature indicates that bromination of DOP and its hydrochloride occurs in different directions involving the benzene ring, C-4 (bromination or oxidation), etc. [2, 4]. Based on this, it is impossible to find any trends in the direction of the reaction although the structures of the products have been proved unambiguously. Furthermore, it is unclear what "purified" and "impure" NBS means [8].

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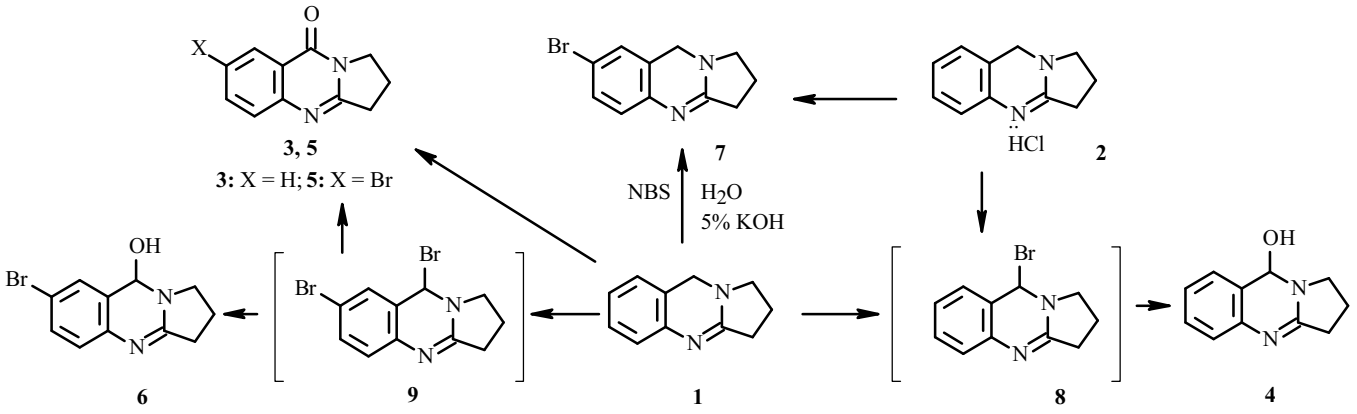
We decided to examine bromination of DOP by NBS in order to find the bromination direction of DOP and its derivatives by NBS, to develop an analytical method for the products using high-performance thin-layer chromatography (HPTLC), and to synthesize peganol and its derivatives.

Bromination of **1** by NBS in $CHCl_3$ (1:1 ratio) gave a mixture of 4-bromo- (**8**) and 4,6-dibromodeoxypeganines (**9**). Treatment of the reaction mixture with NaOH (5%) converted them to peganol and 6-bromopeganol. Compound **3** was also observed in trace quantities. Use of a 1:4 ratio of **1**:NBS and further treatment of the intermediate **5** with NaOH formed only **6**. In contrast with this, bromination of **1** hydrochloride (**2**) in aqueous medium (1:1 ratio) gave 6-bromodeoxypeganine (**7**). Use of a 1:4 ratio of **2**:NBS also changed the reaction direction and formed 6-bromodeoxyvasicinone (**5**).



These data show that DOP and its derivatives are readily brominated or oxidized. We stored compound **4** in methanol at 18-20°C for 70 d in order to determine the degree of mutual conversion and stability of peganol, i.e., its transformation into deoxyvasicinone. We found that it was 16% converted into deoxyvasicinone. Crystalline peganol on standing under these same conditions gave 7% of **3**.

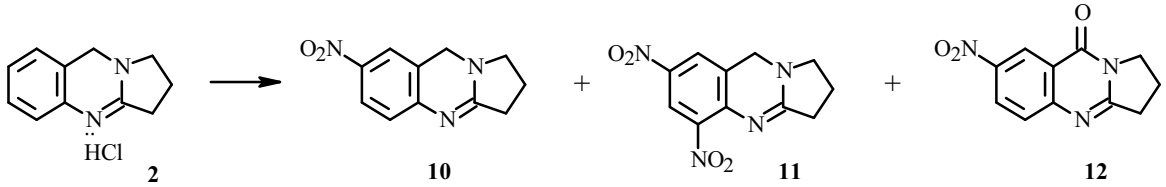
The reaction mixture obtained from deoxypeganine after bromination was left for 70 d and then treated with NaOH (5%) in order to find the ability of DOP to oxidize under the reaction conditions. This produced **5** (30%) and **6** (70%). Storing **6** in methanol under the same conditions formed **5** (45%). These data were obtained by analyzing the reaction mixture using HPTLC.

In contrast with nitration of **1**, where it was shown that one or two nitro groups can be introduced to form 6-nitro- and 6,8-dinitrodeoxypeganines [6, 9], nitration of DOP·HCl by a nitrating mixture (1:1 ratio) gave products (0.57 g from **2**, 1 g) containing 6-nitro-DOP (**10**, 45%), 6,8-dinitro-DOP (**11**, 34%), 6-nitro-DOV (**12**, 16%), and starting **2** (5%).

Performing the reaction with a 1:3 ratio of **2**:nitrating mixture formed a mixture of compounds (0.7 g from **2**, 1 g) including **11** (90%), **10** (7%), and **12** (3%); with a 2:1 ratio, **10** (13%).

We stored 6-nitro-DOP in $CHCl_3$ for 2 d in order to determine the stability of the deoxypeganines upon introducing nitro groups into them. It converted into 6-nitro-DOV (12%). The fraction of oxidation product was 60% upon refluxing in methanol for 4 h. This demonstrated the ease of oxidation of the C-4 CH_2 group in **10**.

Compound **10** was readily oxidized into **12** by $KMnO_4$ in acidic medium upon cooling in an ice bath (68% yield).



We studied the reaction of **11** and NBS under conditions for DOP transformation into peganol in order to prepare 6,8-dinitropeganol (**13**) [4]. However, it was completely transformed into 6,8-dinitro-DOV (**14**). Analogous results were obtained for oxidation of **11** to **13** by $KMnO_4$. In this instance, **14** was also formed. The process passed through intermediate **13**. These results showed that introducing two electron-accepting nitro groups into the 6- and 8-positions facilitates even more the oxidation of the C-4 methylene to a carbonyl.

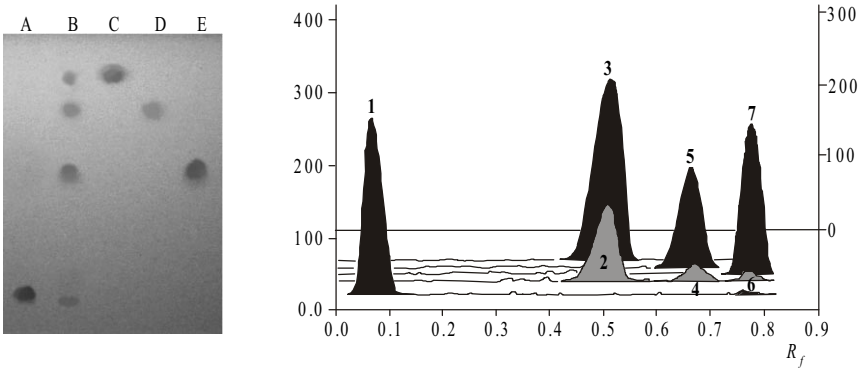


Fig. 1

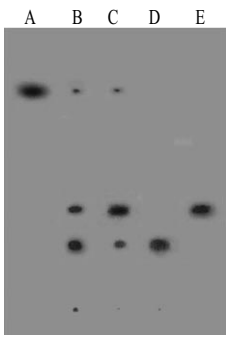
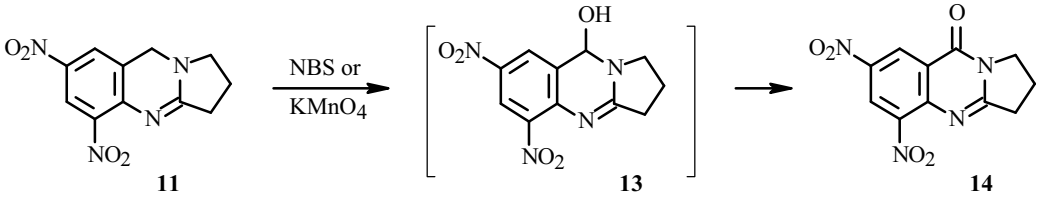


Fig. 2

Fig. 1. Chromatograms of DOP bromination products in $CHCl_3$: DOP (A), bromination products of DOP:NBS (1:1 ratio) (B), DOV (C), 6-bromopeganol (D), peganol (E). Solvent system $CHCl_3$:CH_3OH:$(CH_3)_2CO$:C_6H_{14} (4:1:4:4).
Fig. 2. Chromatograms of DOP nitration products: 6-nitrodeoxyvasicinone (A), nitration products (1:1 ratio) (B), (1:3 ratio) (C), 6-nitrodeoxypeganine (D), 6,8-dinitrodeoxypeganine (E).

We oxidized **2** with $KMnO_4$ under the published conditions in order to convert DOP hydrochloride into peganol [4]. Either **3** or peganol was produced with a 1:1 **2**:$KMnO_4$ ratio depending on the reaction conditions.



11 **13** **14**

The results show that bromination of **1** or its hydrochloride went in different directions. The products in the mixture could not always be isolated pure because of the small content of some of them. Furthermore, their isolation was fraught with losses. Therefore, we used high-performance thin-layer chromatography (HPTLC), which is being used more widely recently along with other analytical methods, in order to determine the qualitative and quantitative composition of the bromination and nitration products. It has several advantages over ordinary TLC because it provides for easy analyses, is rapid (a typical analysis takes less than 20 min), does not require special sample preparation, and uses small amounts of solvents (about 5-10 mL) and cheap aluminum foil. Furthermore, HPTLC can be carried out in several parallel analyses (up to 20 samples) and can separate complicated mixtures, identify them, and measure electronic (UV) spectra of each compound [10].

Figure 1 shows the chromatogram of the DOP bromination products.

It can be seen that bromination formed **4** (62%), **6** (21%), and **3** (7%) and leavings of unreacted starting **1** (10%).

This same method was used to analyze products from mono- and dinitration of DOP. Figure 2 shows the chromatogram of standards and the reaction mixture of the nitration products.

It can be seen that nitration of DOP in a 1:1 ratio formed 6-nitrodeoxypeganine (45%), 6,8-dinitrodeoxypeganine (34%), and a small quantity of 6-nitrodeoxyvasicinone (21%). Use of a 1:3 ratio of reagents gave quantitative contents of them of 90% (**11**), 7% (**10**), and 3% (**12**). The best separation was found for the solvent system benzene:acetone:methanol (5:2:1).

EXPERIMENTAL

IR spectra in mineral oil were recorded on a Perkin—Elmer Model 2000 Fourier spectrometer. PMR spectra were recorded on a UNITY-400+ spectrometer at operating frequency 400 MHz. Samples were prepared in $CDCl_3$ solution with TMS internal standard (0 ppm). Spectra were recorded at room temperature.

Preparation of Samples for HPTLC. An accurately weighed sample or mixture (1 mg) was dissolved in methanol (1 mL). Solution (3 μL) was deposited using a Linomat 5. The width of the tracks was 3 mm; distance between them, 7 mm.

Chromatographic Plates. We used chromatographic plates (Whatman 1 Paper LTD, Germany; Flexible plates TLC AL SIL G/UV). Plates were rinsed with absolute methanol before use.

Solvent Selection. The best separation of bromination products was obtained using $CHCl_3$:CH_3OH: $(CH_3)_2CO$:C_6H_{14} (4:1:4:4); for analysis of nitro derivatives, C_6H_6:$(CH_3)_2CO$:CH_3OH (5:2:1). Elution was carried out in a darkened glass chamber (6 cm distance). Plates were dried in air after elution for 10 min.

Chromatographic plates were scanned on a CAMAG TLC Scanner 3 using the WinCATS program at wavelength 254 nm.

Deoxyvasicinone and deoxypeganine [11]; 6-nitrodeoxyvasicinone [1], and peganol [4] were prepared by the literature methods.

We used commercial NBS without any purification or recrystallization for the studies.

Bromination of deoxypeganine by NBS

a) 1:1 ratio. A solution of DOP (2 g, 11 mmol) in $CHCl_3$ (200 mL) was stirred at room temperature, treated with NBS (2 g, 11 mmol), and stirred at room temperature for 6 h. The resulting precipitate was filtered off, dried in air, and treated with NaOH solution (100 mL, 5%). The precipitate was filtered off (1.5 g) and recrystallized from $CHCl_3$:CH_3OH (2:1). The mixture consisted of **4** (62%), **6** (21), **3** (7), and starting **1** (10); R_f(**4**) 0.49; (**6**) 0.69; (**3**) 0.78; mp 148-152°C.

b) 1:4 ratio. A solution of DOP (2 g, 11 mmol) in $CHCl_3$ (200 mL) was stirred, treated with NBS (8 g, 44 mmol) over 2 h, stirred at room temperature for 6 h, and neutralized with NaOH solution (200 mL, 5%). The resulting precipitate was filtered off (1.52 g), dried, and recrystallized from $(CH_3)_2CO$:$CHCl_3$ (2:1). Yield of **6**, 1.6 g (51.6%), mp 163-1605°C, which agreed with the literature [2].

Reaction of DOP·HCl with NBS

a) 1:1 ratio. A solution of **2** (2.4 g, 11 mmol) in distilled water (100 mL) was treated with NBS (2 g, 11 mmol), stirred at room temperature for 5 h, and neutralized with KOH solution (5%) until the solution was slightly basic. After awhile a precipitate formed and was filtered off and recrystallized from benzene. Yield of **7**, 1.92 g (67.2%), R_f 0.12, mp 173-176°C, which agreed with the literature [11].

b) 1:4 ratio. The reacction was performed analogously to that above from **2** (2.4 g) and NBS (8 g) to produce **5** (1.4 g, 64%), R_f 0.7, mp 158°C, which agreed with the literature [11].

Oxidation of DOP·HCl by $KMnO_4$. A mixture of DOP·HCl (2.1 g, 10 mmol) and aqueous $KMnO_4$ (10%, 1.5 g, 10 mmol) was stirred and treated dropwise with H_2SO_4 solution (10%) at pH 2-3 over 4-5 h. The precipitate was filtered off. The pH was adjusted to 8. The solution was extracted with $CHCl_3$. The extract was dried over anhydrous Na_2CO_3 and filtered. The solvent was evaporated in vacuo. The solid was recrystallized from hexane to afford DOV (1.12 g, 60%), mp 110°C, which agreed with the literature [11].

Oxidation of DOP·HCl by $KMnO_4$ to Peganol (4). An aqueous solution of DOP·HCl (2.1 g, 10 mmol) was placed into a three-necked flask equipped with two dropping funnels and a reflux condenser. A solution of $KMnO_4$ (5%) was added dropwise through the first dropping funnel; H_2SO_4 solution (10%), through the second. The reaction mixture was stirred for 2 h keeping the pH at 2-3 and made basic with NaOH solution (5%). The resulting precipitate was filtered off, washed with water, and recrystallized from methanol to afford **4** (0.87 g, 46%), mp 156-157°C.

Bromination of Peganol. Synthesis of 6-Bromopeganol. Peganol (2 g, 10.6 mmol) was dissolved in $CHCl_3$ (200 mL), stirred, treated with NBS (7.5 g, 42.4 mmol), stirred at room temperature for 6 h, and neutralized with NaOH solution (200 mL, 5%). The resulting precipitate was filtered off, washed with water, dried, and recrystallized from $(CH_3)_2CO$:$CHCl_3$ (2:1). Yield of **6**, 1.8 g (63.4%), mp 163-165°C.

Nitration of DOP·HCl

a) 1:1 ratio of 2:nitrating mixture. DOP·HCl (1 g, 5 mmol) was placed into a three-necked flask equipped with a mechanical stirrer, a dropping funnel, and a reflux condenser, stirred, and treated slowly with conc. H_2SO_4. After complete dissolution, the reaction mixture was cooled to (-5)-(-10)°C, stirred, treated from the dropping funnel with nitrating mixture consisting of HNO_3 (0.4 mL, d = 1.35) and conc. H_2SO_4 (1.2 mL) over 25 min, and stirred at this temperature for 2 h. The temperature was raised to 20-22°C. The mixture was decomposed by gradual addition of water with stirring. The resulting solution was made basic with NaOH solution (5%). The resulting yellow precipitate was filtered off, washed with water, and dried. Yield 0.57 g. Analysis by HPTLC using C_6H_6:$(CH_3)_2CO$:CH_3OH (5:2:1) indicated that the mixture consisted of **10** (45%), **11** (34), **12** (16), and starting **1** (5);

b) 1:3 ratio. The reaction was performed analogously to that above. DOP·HCl (1 g, 5 mmol), HNO_3 (1.2 mL), and conc. H_2SO_4 (3.6 mL) produced a mixture (0.7 g) of compounds consisting of **11** (90%), **10** (7), and **12** (3) [HPTLC data, C_6H_6:$(CH_3)_2CO$:CH_3OH, 5:2:1].

Compound 12. IR spectrum (ν, cm^{-1}): 1462 (NO_2), 1614 (N=C). PMR spectrum (δ, ppm, J/Hz): 4.70 (2H, s, 4-CH_2), 3.40 (2H, t, J = 7.0, α-CH_2), 2.08 (2H, m, β-CH_2), 2.72 (2H, t, J = 8.0, γ-CH_2), 7.83 (1H, d, J = 2.2, H-5), 8.25 (1H, d, J = 2.2, H-7). Mass spectrum (*m*/*z*, %): 262 (84), 246 (9), 232 (16), 215 (43), 185 (41), 169 (100), 157 (40), 149 (39).

c) 2:1 ratio. DOP·HCl (1 g, 5 mmol), HNO_3 (0.2 mL), and conc. H_2SO_4 (1 mL) produced analogously to that above **10** (0.3 g, 13%), mp 187-189°C, which agreed with the literature [6].

Reaction of 10 with $KMnO_4$. A solution of 6-nitro-DOP (1 g, 4.6 mmol) was placed into a four-necked flask (50 mL) equipped with a mechanical stirrer, two dropping funnels, and a reflux condenser, cooled in an ice bath, stirred, and treated from the dropping funnels with $KMnO_4$ solution (5%) and H_2SO_4 solution (10%) over 2 h. The temperature of the reaction mixture was raised to 18-20°C, held at this temperature for 3 h with stirring, neutralized, and extracted three times with $CHCl_3$. The $CHCl_3$ extracts were combined, dried over Na_2SO_4, and evaporated to afford **12** (0.72 g, 68%), R_f 0.85 [C_6H_6:$(CH_3)_2CO$:CH_3OH, 5:2:1]. The physicochemical and spectral data of the product were identical to those published [6, 11].

Reaction of 11 and $KMnO_4$. The reaction was performed analogously to that above. **11** (0.5 g, 1.9 mmol) produced **14** (0.41 g, 78%), mp 192-195°C, R_f 0.9 [C_6H_6:$(CH_3)_2CO$:CH_3OH, 5:2:1].

IR spectrum (ν, cm^{-1}): 1685 (C=O), 1445 (NO_2), 1614 (N=C). PMR spectrum (δ, ppm, J/Hz): 4.22 (2H, t, J = 7.0, α-CH_2), 2.32 (2H, m, β-CH_2), 3.25 (2H, t, J = 8.0, γ-CH_2), 8.74 (1H, d, J = 1.2, H-5), 9.23 (1H, d, J = 1.5, H-7).

Mass spectrum (*m*/*z*, %): 276 (100) $[M]^+$, 260 (3), 246 (28), 200 (11), 186 (20), 172 (18), 163 (15).

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