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Synthesis of d-tryptanthrin

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We recently identified the indolo[2,1-*b*]-quinazoline alkaloid tryptanthrin as the COX-2 inhibitory principle in the ancient dye and medicinal plant *Isatis tinctoria* L. (Brassicaceae) [1]. In various cell based assays as well as with the isolated cyclooxygenases-1 and -2, tryptanthrin shows potent and highly selective inhibition of the COX-2 isoenzyme. Furthermore, the compound strongly inhibits LTB_4 release from human granulocytes and induction of NO synthase [2, 3].

An ESI-LC-MS procedure for the quantitative analysis of tryptanthrin in plant material has been developed and validated [4]. This method uses an external standard, and frequent calibration of the MS is thus required. In view of future analytical needs, a modified protocol using d-tryptanthrin as internal standard was desirable.

First attempts to prepare d-tryptanthrin by deuterium exchange (d-TFA and $D_3PO_4 \times BF_3$) in tryptanthrin were not successful. Even vigorous treatment with $D_3PO_4 \times BF_3$ reagent [5] for 6 d at 80 °C did not result in any noticable exchange, despite a successful treatment of the reference compound daidzein under the same conditions [6–8]. We finally resorted to a three-step synthesis starting from commercially available d-aniline in analogy to reported procedures [9, 10] (Scheme). A first synthesis of d-tryptanthrin (**3**) revealed the risk of hydrogen exchange. While synthesis of d_5-isonitrosoacetanilide (**1**) proceeded smoothly, considerable back exchange occurred during its conversion to d-isatin (**2**) when non deuterated sulphuric acid and water were used. The resulting **2** consisted of a mixture of d_2, d_3 and d_4-isotopomers (3.6, 23.3 and 73.1%, respectively), and **3** contained d_5-, d_6-, d_7- and d_8-isotopomers (1.5, 12.8, 29.2 and 56.5%, respectively), as shown by ESI-MS. ^{1}H NMR spectra revealed that partial exchange occurred at C-2 and C-4 in **2**, and consequently at C-2, C-4, C-8 and C-10 in **3**.

In an improved synthesis, hydrogen exchange in the conversion of **2** to **3** was minimized by the use of D_2SO_4 and D_2O, and the risk of exchange during the other steps was reduced by replacement of H_2O as solvent by D_2O. In this improved synthesis, d-tryptanthrin (**3**) was obtained with an overall yield of 8.7%. The intermediate **2** contained the d_4-, d_3- and d_2-isotopomers (80.0, 18.3 and 1.7%, based on intensity of $[M\text{-}H]^-$ ions in ESI-MS). The ^{1}H NMR spectrum showed signals of the residual H-7 and H-5 at a 2 : 1 ratio which appeared in part as doublets of the *ortho*-coupled signals in d_2-isatin. The final product (**3**) consisted of d_8-, d_7- and d_6-tryptanthrin (77.5, 20.7 and 1.8%, according to signal intensities of $[M+H]^+$ ions). Considering the isotope distribution in d-isatin (**2**), the isotopomer proportions in **3** thus significantly differed from the expected 64.0, 28.8 and 5.9%. We explain the higher isotopic purity achieved by a partial deuterium exchange during the reaction carried out in D_2O. This is supported by the singlet character of the residual H-2, H-4, H-8 and H-10 in the ^{1}H NMR spectrum of **3**. M.p.'s, IR and UV/VIS-spectra of labelled substances were comparable with those of non labelled compounds.

Experimental

1. Characterisation of compounds

Identity and purity of compounds was checked by m.p., HPLC, ESI-MS, UV, IR and ^{1}H NMR.

2. Instruments

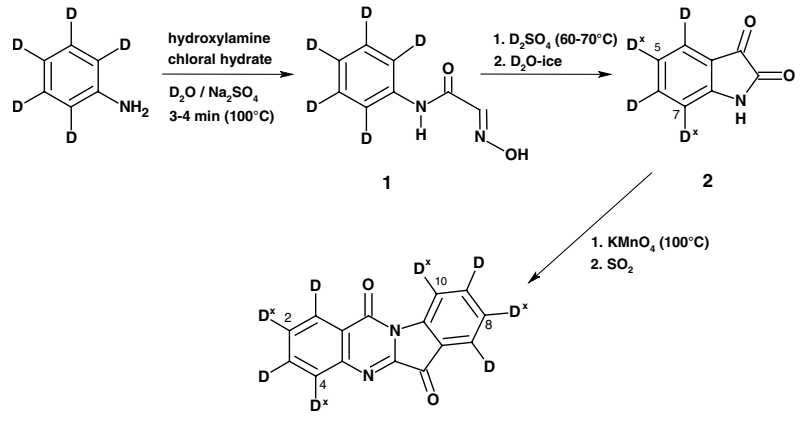
ESI-MS was performed with an API 165 with turbo ion spray interface (Applied Biosystems), connected to a Agilent 1100 HPLC system with DAD-detector. ^{1}H NMR spectra were recorded on a Bruker Advance at 400 MHz. IR spectra were measured on a Specord M 82 (Carl Zeiss, Jena), and UV/VIS spectra with a Beckman DU 640 instrument. MP were determined with a Kofler block.

3. Synthesis

3.1. d-2-(Hydroxyimino)-N-phenylacetamide (d-isonitrosoacetanilide) (**1**)

D_5-aniline (5 g, 0.05 mol, 98% isotope purity) was dissolved in D_2O (32.5 ml, 99.9%) by addition of approx. 2 ml HCl (32%). Chloral hydrate (8.9 g, 0.05 mol) in D_2O (130 ml), sodium sulfate decahydrate (140.4 g, 0.4 mol), and hydroxylamine hydrochloride (12 g, 0.2 mol) in D_2O (54 ml) were added. The solution was quickly heated to ebullition. After 3–4 min, the mixture was rapidly cooled to RT. During this process, white crystals of d-isonitrosoacetanilide appeared. The resulting crystals were collected by filtration, washed with D_2O (3×50 ml) and dried (in vacuo, P_2O_5, 48 h). Yield: 8.4 g (95%), white needles, m.p. 175–179 °C. ESI-MS: m/z (%) = 168.3 ($[d_5\text{-}M\text{-}H]^-$, 100). IR (KBr): $\nu = 3296$, 1666, 1610, 1530, 1456, 1388, 1339, 1234, 1000 cm^{-1}. UV δ_{max} (CH_3OH) 212, 274 nm. ^{1}H NMR (CH_3OD): $\delta = 7.55$ (s, H-8).

Scheme



1 **2** **3**

x Possible positions for hydrogen exchange

3.2. d-1H-Indole-2,3-dione (d-isatin) (**2**)

d-Isonitrosoacetanilide (**1**) (8.4 g, 0.05 mol) was carefully added in small portions to D_2SO_4 (0.15 mol, 8 ml, 99.5%) under stirring and careful control of the reaction temperature which was kept below 70 °C. Upon completion of addition, the reaction mixture was poured on D_2O-ice (100 ml). The red precipitate was filtered, washed with D_2O (3×50 ml) and dried (in vacuo, P_2O_5, 72 h). Yield: 3.0 g (41%), red-orange precipitate, m.p. 193–197 °C. ESI-MS: m/z (%) = 150.2 ($[d_4\text{-M-H}]^-$, 100), 149.4 ($[d_3\text{-M-H}]^-$, 23), 148.4 ($[d_2\text{-M-H}]^-$, <2). IR (KBr): ν = 3432, 3185, 1721, 1604, 1166, 591 cm^{-1}. UV δ_{max} (CH_3OH) 208, 241, 295 nm. ^1H NMR (DMSO-d_6): δ = 6.92 (d, J = 2.8 Hz, H-7), 7.10 (d, J = 2.8 Hz, H-5).

3.3. d-Indolo[2,1-b]-quinazoline (d-tryptanthrin) (**3**)

d-Isatin (**2**) (3 g, 0.02 mol) in D_2O (60 ml) was brought to ebullition, and $KMnO_4$ (3 g, 0.02 mol) was added in small portions and under stirring. While the reaction mixture was kept boiling, SO_2 gas (generated from Na_2SO_3 (50 g) and H_2SO_4 (50%, 150 ml) was bubbled through the solution until a precipitate appeared. The mixture was heated for additional 1–2 min, then cooled to RT. The green-brown precipitate was filtered off, washed with D_2O (3×50 ml) and dried (in vacuo, P_2O_5, 48 h). d-Tryptanthrin was extracted with $CHCl_3$ and recrystallised from CH_3OH. Yield: 540 mg (21.8%), yellow needles, m.p. 268–270 °C. MS (ESI): m/z (%) = 257.2 ($[d_8\text{-M+H}]^+$, 100), 256.3 ($[d_7\text{-M+H}]^+$, 26.6), 255.3 ($[d_6\text{-M+H}]^+$, 2.4). IR (KBr): ν = 3400, 1721, 1684, 1586, 1462, 1351, 1215, 1184, 1160, 1110, 1036, 925, 758, 690, 474 cm^{-1}. UV δ_{max} (CH_3OH) 254, 380, 387 nm. ^1H NMR ($CDCl_3$): δ = 7,45 (s, H-8), 7,69 (s, H-2), 8.05 (s, H-4), 8.65 (s, C-10).

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Pulse polarographic determination of levofloxacin in tablets

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Levofloxacin (**1**), (−)-(*S*)-9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7*H*-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxylic acid hemihydrate, is a quinolone antimicrobial agent which exhibits broad-spectrum *in vitro* bactericidal activities against gram-positive and gram-negative aerobes. **1** is the pure (−)-(*S*)- enantiomer of the racemic drug substance ofloxacin [1].

A few number of studies have been reported for the determination of **1** including first-derivative fluorescence spectroscopy [2], spectrofluorimetry [3], terbium-sensitized luminescence [4] and HPLC [5–9].

The aim of this study was to investigate the optimum polarographic conditions for the determination of **1** based on the reduction of the quinoline ring and to apply the method to pharmaceutical preparations. The optimum polarographic parameters were elucidated and **1** was determined using direct current (DC), differential pulse (DP), superimposed constant amplitude pulse (SCAP) and superimposed increasing amplitude pulse (SIAP) polarographic techniques in tablets. The experiments were conducted in the aqueous supporting electrolyte containing 0.2 mol/l KCl 10% v/v MeOH and 0.2 mol/l buffer solution.

A two step polarogram was obtained at pH values higher than 8 and lower than 5. The morphology of this curves dramatically changes depending on small changes in pH. A single step perfect polarogram was obtained at pH values between 5 and 8. Quantitative determination of **1** is not possible outside of this range. In spite of low limiting current, at pH between 5-8, suitable curves were obtained in the view of quantitative evaluation. Therefore pH 7.12 has been chosen as a working value. The stability of **1** in pH 7.12 buffer solution was examined. The prepared solutions give the same polarograms during a week time.

The process was found to be irreversible according to the criteria of Birke et al. [10], and the control of the polarographic current was diffusional at pH 7.12. The effect of temperature was investigated in the range of 15–45 °C. The variation of limiting current was found to be 1.15 μA/°C. The variation confirms that the polarographic current is diffusion controlled [11].

The calibration studies were performed using DC, DP, SCAP and SIAP polarographic techniques. The polarograms are demonstrated in the Fig.

The variation of **1** concentration was investigated in the range of $1 \times 10^{-4} - 5 \times 10^{-4}$ mol/l. The equations were calculated measuring the current at the maximum of the waves or peaks.

At −1340 mV for DC
$[i_{lim}(\mu A) = 1812.3\ C(mol/l) - 0.048\ r = 0.9998]$
At −1300 mV for DP;

$[i_{lim}(\mu A) = 1167.8\ C(mol/l) - 0.032\ r = 0.9997]$
At − 1360 mV for SIAP;

$[i_{lim}(\mu A) = 3630.6\ C(mol/l) - 0.017\ r = 0.9996]$
At the peak maximum for SCAP;

$[i_{lim}(\mu A) = 3570.6\ C(mol/l) - 0.112\ r = 0.9994]$