SHORT COMMUNICATIONS

SYNTHESIS AND NITRATION OF SOME SUBSTITUTED 2-(2'-FURYL)CINCHONINIC ACIDS

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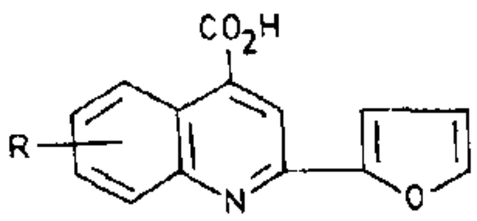
QUINOLINE ring structure is found in medicinal plant alkaloids such as cinchonine and quinine which are used for the treatment of malaria[1]. This prompted the development of synthesis of a wide range of antimalarials possessing quinoline nucleus. As a result, synthetic antimalarials such as chloroquine, plasmoquine etc. are now being used in chemotherapy[2]. Certain halogenated derivatives of 8-hydroxy quinolines exhibit bactericidal and fungicidal activities. Some of them also act as amoebicides and tumour inhibiting agents[3,4]. Styrylquinolines and their quaternary iodides have been the subject of extensive screening for tumour-inhibiting properties, treatment of leukemia and for bactericidal and fungicidal activities[4]. Cinchophen, 2-phenylcinchoninic acid is used as analgesic and its hydrochloride finds application as antipyretic and uricosuric drug[5].

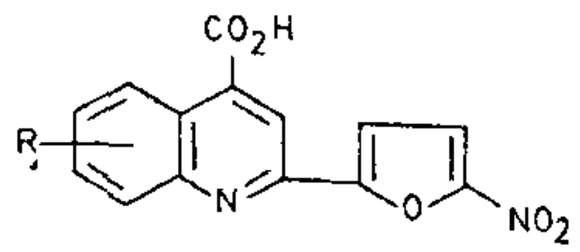
Prompted by these observations and as a part of our general search[6] for potential antibacterial agents containing nitrofuran ring it was considered worthwhile to synthesize and study the nitration of a series of substituted 2-(2'furyl)cinchoninic acids and the biological activities of the resulting nitrofuran derivatives. This is described in the present paper.

The reaction of various substituted isatins with 2-acetylfuran under the conditions of Pfitzinger reaction[7] gave substituted 2-(2'-furyl)cinchoninic acids(I) which are characterized by elemental analysis, IR, NMR and mass spectral data. These compounds when nitrated gave moderate to good yields of mononitro derivatives. These mononitro derivatives are now characterized as 2-(5'-nitro-2'-furyl)cinchoninic acids (II) by elemental analysis, NMR and mass spectral data.

The nitrated compounds are shown to be mononitro derivatives by elemental analyses and mass spectra. The mass spectrum of (Ie) showed molecular ion peak at m/e 307 while its nitrated product showed a molecular ion peak at m/e 352, thus confirming the mononitration. The fragment peaks are observed at m/e 322, 294 and 278 which correspond to the ions obtained due to the successive loss of NO, NO and CO and NO and CO_2 from the molecular ion. The formation of these fragment ions can be rationalized by the fragmentation of a nitrofuran ring during electron impact studies. In chlorinated derivatives the isotope peaks corresponding to both ^{35}Cl and ^{37}Cl isotopes are also observed.



I



II

That the nitro group has entered the 5'-position of the furyl moiety is also evident by the examination of the NMR spectra of 5-methyl-2(2'-furyl)cinchoninic acid (Ia) and its nitrated product. The signal due to the 4'-proton of furan appeared as a quartet at δ, 6.46 indicating the typical AMX coupling pattern of monosubstituted furan ring. This quartet disappeared in the NMR spectrum of the nitrated product (IIa). Further the signal due to 4'-proton of furan moiety in (IIa) shifted downfield and got mingled with the multiplet due to the aromatic protons at δ, 7.44 thereby confirming the nitration of the 2'-furyl substituent.

The nitrofuryl cinchoninic acids (IIa-e) thus synthesized were subjected to antibacterial screening against *S. aureus*, *B. subtilis*, *E. coli*, *P. aeruginosa* and antifungal screening against *Candida albicans* employing the disc-diffusion technique on agar plates. Among the compounds tested, compound (II-e) showed highest inhibition (17 mm) against *S. aureus* at 10 μg/ml concentration while (II-d) showed weaker inhibition (9 mm) against the same bacteria. The other compounds did not possess significant activity against the micro-organisms tested.

The melting points of the new compounds were determined by the capillary method and are uncorrected. The IR spectra (nujol mull) were recorded on an infrared spectrophotometer (Perkin Elmer), NMR spectra of some selected compounds were recorded on a 60 MHz NMR spectrometer using DMSO-d_6 as solvent and tetramethyl silane as internal standard. The mass spectra of some com-

Table 1 *Characterization data of cinchoninic acids (I a-e) and their nitro derivatives (II a-e)*

Compound	R	Yield(%)	Colour	M P.°C	Molecular formula*	$\gamma_{C=O}$ (cm^{-1})
Ia	6-Methyl	63	Pale yellow flakes	254–55[f]	$C_{15}H_{11}NO_3$	1720
Ib	6-Nitro	46	Reddish brown cubes	330–33[f]	$C_{14}H_8N_2O_5$	–
Ic	5-Chloro	62	Yellow flakes	245–47[f]	$C_{14}H_8ClNO_3$	1720
Id	7-Chloro	57	Dark yellow micro needles	282–83[f]	$C_{14}H_8ClNO_3$	1720
Ie	6,8-Dichloro	75	Yellow micro needles	276–77[f]	$C_{14}H_7Cl_2NO_3$	1700
IIa	6-Methyl	70	Lemon yellow micro needles	270–72[g]	$C_{15}H_{10}N_2O_5$	1740
IIb	6-Nitro	67	Dark brown cubes	>360[g]	$C_{14}H_7N_3O_7$	1730
IIc	5-Chloro	73	Brownish micro needles	170–72[g]	$C_{14}H_7ClN_2O_5$	1740
IId	7-Chloro	86	Lemon yellow flakes	247–48[f]	$C_{14}H_7ClN_2O_5$	1730
IIe	6,8-Dichloro	76	Light brown micro needles	295–96[f]	$C_{14}H_6Cl_2N_2O_5$	1730

Solvent for crystallization : f=glacial acetic acid, g=DMF; *Satisfactory analytical data were obtained for all compounds.

pounds were recorded on a spectrometer (JEOL, JMS-D300).

5-Methyl isatin[8], 4 and 6 chloro isatins[9] were prepared by the methods reported earlier. 5,7-Dichloro and 5-nitro isatins were obtained commercially and were used after recrystallization.

General procedure for the synthesis of 2-(2'-furyl) cinchoninic acids (I-e)

A solution of sodium hydroxide (2 g, 50 mmol) and 5-methyl isatin (1.61 g, 10 mmol) in water (25 ml) was heated on a boiling water bath for 45 min and a clear solution is obtained. 2-Acetylfuran (1.10 g, 10 mmol) was added to this solution in small portions with occasional shaking and the heating was continued for 2.5 h following the addition of 2-acetylfuran. After the reaction, the mixture was chilled in an ice bath and the contents were filtered through a sintered glass crucible (G-4). The precipitate of the sodium salt was dissolved in water (100 ml) and the resulting solution was acidified with glacial acetic acid wherein the 2-(2'-furyl)cinchoninic acid separated as a yellowish precipitate. This was collected by filtration, washed, drained and air-dried. It was recrystallized from glacial acetic acid to yield 2-(2'-furyl)-6-methyl quinoline-4-carboxylic acid (I-a) as light yellow crystals. Similarly the other substituted quinoline-4-carboxylic acids (I b-e) were also synthesized. The physical constants, colour, yield and IR data are given in table 1.

General procedure for the synthesis of 2-(5'-nitro-2'-furyl) cinchoninic acid (II a-e)

The powdered cinchoninic acid Ia (2.5 g, 10 mmol) was added in small portions to well stirred conc. sulphuric acid (15 ml) cooled and maintained at 0°C. When the addition was complete a cold solution of conc. nitric acid (1.5 ml) in conc. sulphuric acid (1.5 ml) was added dropwise to the cold solution of cinchoninic acid in sulphuric acid. Stirring was continued for 1 h at 0–5°C after adding the nitrating mixture. The contents of the reaction mixture were poured onto crushed ice (200 g) with stirring. The crude precipitate was collected by filtration, washed with water and recrystallized from glacial acetic acid to yield lemon yellow microneedles of 2-(5'-nitro-2'furyl)-6-methyl-quinoline-4-carboxylic acid (II a). The other cinchoninic acid (I b-e) were similarly nitrated and the physical constants, colour, yield and IR data of compounds II a-e are tabulated in table 1.

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1. Yates, F. S., In: *Comprehensive heterocylic chemistry*, (eds) A. R. Katritzky and C. W. Rees, Pergamon Press, New York, 1984, Vol. 2, p. 512.
2. Acheson, R. M., *An introduction to the chemistry of heterocyclic compounds*, 2nd edn, John Wiley, New York, 1967, p. 266.
3. Markov, R., Pavlova, A. and Vlakhova, B., (Bulg) *Tr. Nauchnoizshed. Khim. Farm. Inst.*, 1972, **8**, 135, *Chem. Abstr.* **79**, 53154.

4. Claret, P. A., In: *Comprehensive organic chemistry*, (eds) D. H. R. Barton and W. D. Ollis, Pergamon Press, New York, 1979, Vol. 4, p. 198.
5. *The Merck Index*, 9th edition, (ed.) M. Windholz, Merck and Co, Inc., New Jersey, 1976, p. 2278.
6. Holla, B. S., Kalluraya, B. and Sridhar, K. R., *Curr. Sci.*, 1987, **56,** 236.
7. Burch, H. A., *J. Med. Chem.*, 1969, **12,** 535.
8. *Organic synthesis coll.*, John Wiley, New York, 1967, Vol. 1, p. 327.
9. Senear, A. E., Sargent, H., Mead, J. R. and Roefli, J. B., *J. Am Chem. Soc.*, 1946, **68,** 2695.

EFFECT OF FEEDING FENUGREEK *(TRIGONELLA FOENUM GRACECUM)* LEAVES ON FAECAL EXCRETION OF TOTAL LIPIDS AND STEROLS IN THE NORMAL ALBINO RABBITS

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SPICES are important in human dietaries to enhance the sensory quality of food. They also exhibit a wide range of beneficial physiological and biochemical effects in the body. Fenugreek (*Trigonella foenum gracecum*), commonly known as 'methi' a leguminous aromatic plant, is described as a medicinally important plant in the Ayurvedic system of medicine[1]. Seeds of Fenugreek have hypocholesterolemic and antidiabetic activities[2–6] and their leaves have hypocholesterolemic activity[7]. The present study was undertaken to determine the effect of incorporating Fenugreek leaves in the diet of normal rabbits, on faecal excretion of total lipids and sterols.

Fresh green leaves of 'methi' lost 90% of its weight (due to moisture) when dried at 20° to 22°.

Sixteen normal adult male albino rabbits were maintained on rabbit's feed (Hindustan Gold Mohr) for a month. Before starting the experiments, faecal matter of animals starved for 12 h was collected for 24 h and analysed for total lipids and sterols, for 2 consecutive weeks to ensure an almost constancy of the above parameters with respect to diet. The rabbits were divided into two groups, experimental and control groups, each consisting of eight rabbits.

Thereafter, feeding of experimental diet (containing 1 g of dried green leaves which were boiled in water along with basal diet) was started and continued for 8 weeks. The rabbits of control group were kept only on control diet without the leaf addition. The animals had free access to food and water. Their daily food consumption was from 95 to 100 g. The food intake was essentially similar in both groups. Faecal matter of animals starved for 12 h was collected at the end of 1st, 2nd, 4th and 8th weeks, each time, and analysed for total lipids[8] and sterols[8,9] in the 24 h faecal excretion. Body weight was recorded at the beginning and end of the 1st, 2nd, 4th and 8th weeks. The difference between the groups was evaluated by Student's *t* test.

Analysis of the faeces after ingestion of cooked, dried leaves of Fenugreek 1 g/day for total lipids and sterols in 24 h faecal excretion, revealed increase in the faecal total lipids as well as sterols excretion, in the duration of eight-week feeding. Maximum increase in the levels of lipids and sterols was observed after the 8th week (table 1).

Biochemical studies have been reported regarding the hypocholesterolemic[2–5], hydrocholagoguic[10] and hypoglycemic[5,6] activities of the Fenugreek seeds. We have reported[7] that fresh green leaves of *Trigonella foenum gracecum*, when incorporated in the diet of normal rabbits, caused lowering in serum

Table 1 *Effect of ad libitum feeding fenugreek (Trigonella foenum gracecum) green leaves (1 g/day) on dry weight basis on faecal total lipids and sterols excretion per 24 h in normal rabbits (values are mean ± SD, expressed in mg/24 h faecal excretion)*

Diet	Control+ group (no. leaf addition)	Experimental* Group addition of leaf 1st week	2nd week	4th week	8th week
		Cooked leaves			
Body weight (kg)	1.4820 ±0.131	1.5000 ±0.128 (1.21)	1.5240 ±0.133** (2.83)	1.5570 ±0.142 * (5.06)	1.5950 ±0.137 (7.62)
Faecal lipids	168.35 ±23.30	204.45 ±24.41* (21.44)	233.45 ±43.24 (38.67)	287.04 ±38.47* (70.50)	345.90 ±42.16* (105.45)
Faecal sterols	64.59 ±6.72	72.84 ±7.30 (12.77)	78.62 ±8.17* (21.72)	83.38 ±7.93* (29.09)	105.40 4.37 (63.18)

*Sample size in each case was 8, Figures in parentheses indicate per cent change. $P<0.001$; ** $P<0.01$; * $P<0.05$.