[CONTRIBUTION FROM THE CONNAUGHT LABORATORIES, UNIVERSITY OF TORONTO]

Chemotherapeutic Study of *p*-Nitrobenzoyl- and Related Compounds

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Evidence has been presented by Mayer and Oechslin[1] and by Rosenthal and Bauer[2] that *p*-nitrobenzoic acid and other simple nitro compounds may exert a slight activity in infections with pathogenic cocci. As part of a chemotherapeutic study of nitro compounds,[3] a number of cyclic mono- and poly-hydroxy compounds as well as mono- and di-amino compounds have been benzoylated with *p*-nitrobenzoyl chloride either in pyridine or in presence of aqueous alkali. Where mixtures were obtained they were largely separated by fractional crystallization. In some instances, the 3,5-di-nitrobenzoyl derivatives were prepared for comparison. Some typical experiments are described in the experimental section and the properties of the compounds are recorded in Table I. Among the sixteen new compounds listed is the *p*-nitrobenzoyl ester of inositol,[4] which was prepared by heating *p*-nitrobenzoyl chloride with dry inositol.

Sulfanilamide Derivatives.—Of the possible *p*-nitrobenzoyl derivatives of sulfanilamide, the N^1- as well as the N^4-*p*-nitrobenzoylsulfanilamide have been mentioned previously,[5,6] the N^1-compound having been described as inactive.[6] Both substances, as well as the so far unknown N^1,N^4-di-*p*-nitrobenzoyl-sulfanilamide were synthesized (Table II). This di-compound, though it is, like the N^4-substituted product, without chemotherapeutic activity, proved to be useful as an intermediate in the synthesis of N^1-*p*-nitrobenzoyl-sulfanilamide. For preparing the latter substance, the method described by Crossley and coworkers[5] was first used.

A simpler method of preparing this substance in the laboratory consists in condensing one mole of sulfanilamide with two moles of *p*-nitrobenzoyl chloride, followed by hydrolysis of the resulting N^1,N^4-di-*p*-nitrobenzoyl-sulfanilamide (see Experimental).

Similarly the N^1-benzoylsulfanilamide was prepared from sulfanilamide and benzoyl chloride with formation of N^1,N^4-di-benzoylsulfanilamide as an intermediate. The N^1-benzoylsulfanilamide has previously been prepared from N^4-acetyl-sulfanilamide.[5,7] The purpose of our preparing this compound was to compare its biological action with that of N^1-*p*-nitrobenzoylsulfanilamide.

Biological Properties

The compounds described were tested in experimental infections of mice with hemolytic streptococci, pneumococci and meningococci. The tests confirmed the slight antistreptococcal and antipneumococcal activity of *p*-nitrobenzoic acid[1] which also had a very slight action upon meningococci. None of the compounds listed in Table I showed a therapeutic activity comparable to that of active sulfonamides. The *p*-nitrobenzoyl ester of cyclohexanol exhibited a slight activity against streptococci but not against pneumococci as previously reported.[1]

The mixture of the mono- and the di-*p*-nitrobenzoyl ester of 4-hexylresorcinol, when given to mice subcutaneously, exhibited antipneumococcal properties, not shown by the unsubstituted 4-hexylresorcinol.[8]

None of the 3,5-di-nitrobenzoyl derivatives possessed any therapeutic activity.

In the sulfonamide series (Table II), the introduction of a benzoyl or *p*-nitrobenzoyl group into the N^1-position of the sulfanilamide molecule leads to compounds with very low toxicity. The N^1-*p*-nitrobenzoyl-sulfanilamide proved, according to a finding by Dr. K. Sternbach,[9] to be very effective in meningococcal infections in mice. This activity is interesting in view of the chemical constitution of this substance, according to which *p*-aminobenzoic acid may be formed in the animal body. Under the experimental conditions, however, not enough of the inhibiting substance was formed to interfere with the antimeningococcal action of the sulfonamide.

The corresponding N^1-benzoyl compound showed a lower antimeningococcal activity, but was specifically active in pneumococcal infections.[10] This is in agreement with a report by Dvornikoff.[7]

The di-substituted N^1,N^4-compounds were completely inactive.

(1) Mayer and Oechslin, *Arch. intern. pharmacodynamie*, **62**, 211 (1939).

(2) Rosenthal, Bauer and Elvove, *Pub. Health Rep.*, **54**, 1317 (1939).

(3) Siebenmann and Schnitzer, *Can. Pub. Health J.*, **74**, 321 (1941).

(4) The inositol was kindly supplied by Dr. H. O. L. Fischer (Banting Institute) to whom we are indebted for suggesting the preparation of this ester.

(5) Crossley, Northey and Hultquist, THIS JOURNAL, **61**, 2950 (1939).

(6) Northey, *Chem. Rev.*, **27**, 85 (1940), (revised reprint) "Structure and Chemotherapeutic Activities of Sulfanilamide Derivatives."

(7) Dvornikoff (Monsanto Chem. Co.) U. S. Patent 2,240,496 (May 6, 1941).

(8) Leonard, *J. Am. Med. Assoc.*, **83**, 2005 (1924).

(9) All antimeningococcal tests were carried out by Dr. K. Sternbach under a grant of the Banting Research Foundation (1940–41).

(10) This activity is considerable, though less marked than for sulfadiazine. In a recent report, Laeuger and Martin (*Schweiz. med. Woch.*, 1943, p. 399) make the erroneous claim that N^1-benzoylsulfanilamide is inactive. They attribute a marked antipneumococcal activity of N^1-3,5-di-methyl-benzoylsulfanilamide ("Irgafen") entirely to the presence of the methyl groups.

TABLE I

NITROBENZOYL DERIVATIVES OF HYDROXY AND AMINO COMPOUNDS[a]

Compounds	Empirical formula	Melting range, °C. (uncor.)	Appearance	Solubility	N, % Calcd.	N, % Found
p-Nitrobenzoyl esters:						
Catechol monoester	$C_{13}H_9O_5N$	151–152	Prisms	S. acetone, dil. NaOH	5.40	5.51
Catechol diester	$C_{20}H_{12}O_8N_2$	162–165	Needles	S. acetone	6.85	6.78
Hydroquinone monoester	$C_{13}H_9O_5N$	190–194[e]	Needles	S. acetone, dil. NaOH	5.40	5.62
Hydroquinone diester	$C_{20}H_{12}O_8N_2$	252–257[d]	Short prisms	S. acetone	6.85	6.95
Resorcinol monoester	$C_{13}H_9O_5N$	175–177	Short prisms	S. acetone, dil. NaOH	5.40	5.60
Resorcinol diester	$C_{20}H_{12}O_8N_2$	185–186	Fine needles	S. acetone	6.85	6.72
Pyrogallol monoester	$C_{13}H_9O_6N$	193–197	Pale yellow powder	S. acetone, dil. NaOH	5.09	5.25
Pyrogallol triester	$C_{27}H_{15}O_{12}N_3$	229–231	Short needles	S. hot acetone, dioxane	7.32	7.56
4-Hexylresorcinol monoester	$C_{19}H_{21}O_5N$	M. p. of mixture 60–72	Oil crystallizing on long standing	S. ether, acetone	4.08	4.97
4-Hexylresorcinol diester	$C_{26}H_{24}O_8N_2$			Benzene Olive oil	5.69	
Cyclohexanol ester	$C_{13}H_{15}O_4N$	51.5–52.5	Prisms	S. alc., warm olive oil	5.61	5.85
Inositol hexaester[b]	$C_{48}H_{30}O_{24}N_6$	310–315	Needles	Sl. s. hot nitrobenzene	7.82	8.08
3,5-Dinitrobenzoyl ester of Cyclohexanol	$C_{13}H_{13}O_8N_2$	109–111	Short needles	Recr. hot alc.	9.55	9.69
p-Nitrobenzoyl-						
-N-morpholine	$C_{11}H_{12}O_4N_2$	101–106	Prisms	S. hot water, methyl alc.	11.88	12.16
-N-piperidine	$C_{12}H_{14}O_3N_2$	115–118	Needles	Recr. alc.-w	12.16	12.00
-N-cyclohexylamine	$C_{13}H_{16}O_3N_2$	203–204	Needles	Recr. alc.-w	11.30	11.45
3,5-Dinitrobenzoyl-						
-N-morpholine	$C_{11}H_{11}O_6N_3$	184–187	Rhomb. prisms	Acetone, hot alc.	15.00	15.00
-N-piperidine	$C_{12}H_{13}O_6N_3$	143–144.5	Reg. prisms	Acetone, hot alc.	15.05	15.30
Di-*p*-nitrobenzoyl-						
-N,N[1]-piperazine	$C_{18}H_{16}O_8N_4$	318	Short needles	Sl. s. hot pyridine	14.60	14.75

[a] Unless otherwise stated, the compounds listed were prepared by condensation of the hydroxy or amino compounds with the corresponding nitrobenzoyl chloride in pyridine solution. [b] Prepared by heating a mixture of dry *p*-nitrobenzoyl chloride (0.065 mole) and dry inositol (0.01 mole) at 180–200° for forty minutes. The reaction product was washed with acetone and recrystallized from nitrobenzene–alcohol. [c] Izmailskii and Belotsvetow, *J. Gen. Chem.* (U. S. S. R.) II, 650 (1941), m. p. 192–193.5°. [d] Izmailskii and Belotsvetow, *ibid.*, m. p. 263–264.5°. [e] Prepared by Schotten–Baumann reaction (5% NaOH).

TABLE II

SULFANILAMIDE DERIVATIVES

-Sulfanilamide	Formula	M. p., °C. (uncor.)	Appearance	Solubility, recr.	Analyses, % N Calcd.	N Found	S Calcd.	S Found
N[1]-*p*-Nitrobenzoyl-	$NH_2C_6H_4SO_2NHCOC_6H_4NO_2$	218–219[a]	Orange needles	Acetone–alc.–w.	13.10	13.65	9.94	9.65
N[4]-*p*-Nitrobenzoyl-	$NO_2C_6H_4CONHC_6H_4SO_2NH_2$	260	Fine needles	Acetone–alc.–w.	13.10	13.27	9.94	9.80
N[1],N[4]-Di-*p*-nitrobenzoyl-	$NO_2C_6H_4CONHC_6H_4SO_2NHCOC_6H_4NO_2$	268 (dec.)	Long prisms	Hot acetone	11.92	12.04	6.80	6.70
N[1]-Benzoyl-	$NH_2C_6H_4SO_2NHCOC_6H_5$	178–180[b]	Long prisms	Hot alc.	10.13	10.27	11.60	11.14
N[1],N[4]-Dibenzoyl-	$C_6H_5CONHC_6H_4SO_2NHCOC_6H_4$	252[c] dec.	Prisms	Acetone–alc.–w.	7.36	7.52	8.43	8.32

[a] Crossley and co-workers[5] m. p. 235–240°. [b] Crossley and co-workers[5] m. p. 181.2–182.3°; Dvornikoff[7] m. p. 178–179°. [c] Dewing, Gray, Platt and Stephenson, *J. Chem. Soc.*, 239 (1942), m. p. 268–270°.

Experimental

The general method of synthesis is illustrated by detailed directions for the preparation of some representative compounds. The analytical data are found in Tables I and II. All melting points are uncorrected.

Cyclohexanol-*p*-nitrobenzoyl Ester.—To an excess of 20 g. (0.2 mole) of cyclohexanol dissolved in 100 cc. of pyridine, 18.6 g. (0.1 mole) of *p*-nitrobenzoyl chloride was added in small portions with constant stirring, keeping the temperature below 20°. The mixture was then boiled on reflux for fifteen minutes. On standing overnight, a crystalline mass formed, which was transferred into 700 cc. of water. The suspension of the crude ester was boiled for ten minutes. The ester, which on heating became an oil, resolidified on cooling the mixture with ice. The product was filtered off, washed with water and dried *in vacuo*; yield 16 g. (64%, calculated on the basis of *p*-nitrobenzoyl chloride used); m. p. 47–50°. The ester was dissolved in 700 cc. of hot alcohol. The alcoholic solution was decolorized with charcoal. To the warm filtrate 350 cc. of hot water was added. On cooling, the pure ester crystallized; yield 14 g., m. p. 51.5–52.5°. This ester is insoluble in diluted sodium hydroxide but dissolves in warm olive oil.

Resorcinol-*p*-nitrobenzoyl Esters.—To 22 g. (0.2 mole) of resorcinol, dissolved in 75 cc. of pyridine, 20.4 g. (0.11 mole) of *p*-nitrobenzoyl chloride in 100 cc. of pyridine were added. The mixture was then heated (100°) for thirty minutes, cooled to 40° and poured into 1500 cc. of ice-water. The white crystalline precipitate which formed was suction filtered, washed with water and dried at 100° (18 g., m. p. 152–170°). This product, a mixture of the mono-

and the di-ester, was dissolved in 150 cc. of hot acetone–alcohol (1:1) and filtered, leaving behind a small precipitate which was discarded.

Di-ester.—From the cooled filtrate some resorcinol-di-*p*-nitrobenzoyl ester (fraction a) crystallized, which was filtered, washed with water and dried (100°); yield 3.5 g., m. p. 183–185°. After recrystallization from acetone–water, it melted at 185–186°. This ester is insoluble in cold 10% sodium hydroxide.

Mono-ester.—The filtrate of fraction (a) was poured into 1200 cc. of ice-water. After standing overnight, the white crystalline precipitate (fraction b) which had formed was separated, washed and dried *in vacuo*, yielding 12.4 g. of the crude mono-ester, m. p. 174–175°. It was purified by means of fractionated crystallization by dissolving it in 500 cc. of hot acetone–alcohol (1:1). Addition of 200 cc. of water to this clear solution did not lead to the formation of a precipitate until the mixture was ice cooled. After standing for two hours, a small amount of impure di-ester (2 g.) was filtered off. The filtrate was poured into 1 liter of ice-water. The white crystalline precipitate which formed was separated; yield 8.9 g. of pure mono-ester, m. p. 175–177°. The mixture of this ester with fraction (a) melted at 165–170°. In contact with diluted sodium hydroxide, the monoester immediately turns orange-brown and slowly dissolves to form a clear and almost colorless solution, which on standing becomes dark brown.

The di-ester (fraction a) can be obtained with better yield by using for the condensation an excess of *p*-nitrobenzoyl chloride as described for the preparation of the pyrogallol-tri-*p*-nitrobenzoyl ester.

Pyrogallol-*p*-nitrobenzoyl Esters

Mono-ester.—To a solution of 12.6 g. (0.1 mole) of pyrogallol in 100 cc. of pyridine, 18.6 g. (0.1 mole) of solid *p*-nitrobenzoyl chloride was added while stirring. The mixture was heated for thirty minutes (100°), then poured into 800 cc. of ice-water. A yellow oil separated out. The mixture was acidified with hydrochloric acid and extracted three times with 200 cc. of ether. The pooled ether extracts were dried with sodium sulfate. The ether was evaporated, leaving behind a yellow oil which crystallized on standing overnight; yield 16 g. (38%), m. p. 190–195° The crude mono-ester was dissolved in 150 cc. of acetone. After treatment with charcoal, the yellow filtrate was poured into 4 volumes of ice-water. On standing, a yellow crystalline precipitate separated out which, after washing with water and drying *in vacuo*, had a melting point of 193–197°. After recrystallization from alcohol–water, the melting point remained unchanged. The dry monoester is stable. It dissolves in diluted sodium hydroxide with light brown color, which on contact with air becomes dark.

Tri-ester.—To a well-stirred solution of 18.6 g. (0.1 mole) of *p*-nitrobenzoyl chloride in 100 cc. pyridine, 4.2 g. (0.033 mole) of pyrogallol, dissolved in 20 cc. of pyridine were added. The mixture was then heated (100°) for fifteen minutes and after standing overnight was poured into 800 cc. of ice-water. The crystalline precipitate which formed was washed with water and dried *in vacuo*, yielding 16.5 g. (91%), m. p. 222–227°. After recrystallization from hot dioxane, the melting point was 225–230°. A second recrystallization left the melting point unchanged. The tri-ester is colorless. It is insoluble in cold 10% sodium hydroxide.

Hexylresorcinol-*p*-nitrobenzoyl Esters.—9.7 g. of 4-hexylresorcinol (0.05 mole) was dissolved in 30 cc. of pyridine. To this a solution of 18.6 g. (0.1 mole) of *p*-nitrobenzoyl chloride in 100 cc. of pyridine was added. After heating the mixture for one hour (100°), it was poured into 700 cc. of ice-water. The mixture, containing an oily product, was made alkaline to phenolphthalein by means of sodium hydroxide and then extracted three times with 200-cc. portions of ether. The combined ether extracts were first washed with 0.5% sodium hydroxide, then with 1% hydrochloric acid to remove traces of pyridine and finally with water. After treatment with charcoal, the ether filtrate was dried with sodium sulfate. The ether was distilled off, leaving behind an amber-colored oil (25 g.), which after ten days of standing in a desiccator formed a crystalline mass. This product melted between 60 and 72°. It was insoluble in water and diluted sodium hydroxide. The analysis is indicative of a mixture of the mono- and the di-*p*-nitrobenzoyl ester of 4-hexyl-resorcinol. Calcd. for mono-ester $C_{19}H_{21}O_5N$: C, 66.46; H, 6.12; N, 4.08. Calcd. for di-ester $C_{26}H_{24}O_8N_2$: C, 63.42; H, 4.88; N, 5.69. Found: C, 64.70; H, 5.23; N, 4.97. A 5% solution of this product in olive oil was used for the biological tests.

Sulfanilamide Derivatives

N^1,N^4-Di-*p*-nitrobenzoyl-sulfanilamide (I).—To a well stirred solution of 43 g. (0.23 mole) of *p*-nitrobenzoyl chloride in 320 cc. of pyridine, 19.0 g. (0.11 mole) of sulfanilamide dissolved in 60 cc. of pyridine was added, keeping the temperature below 30°. The mixture was then heated on the water-bath for one hour, cooled to 40° and poured into 2 liters of ice-water. The white precipitate which formed was washed with water and dried at 100°; yield 49 g. (95%), m. p. 262° (dec.). After recrystallization of a small sample from boiling acetone, the melting point was 268° (dec.) and remained unchanged by further recrystallization. The mixed melting point with N^4-*p*-nitrobenzoylsulfanilamide (III) was 245–250°.

N^1-*p*-Nitrobenzoyl-sulfanilamide (II).—47 g. (0.1 mole) of (I) was suspended in 400 cc. of water, placed in a 1-liter flask. The well-stirred mixture was heated to boiling. Then 40 cc. of a 30% sodium hydroxide solution was added and the stirring and heating (over a small flame) continued for a short time until all the di-compound was dissolved. The solution was then boiled on reflux for thirty minutes, cooled rapidly to 15° and 200 g. of crushed ice added. The mixture was vigorously stirred while slowly adding enough 2.5 normal hydrochloric acid to bring the *p*H to 5.5 (glass electrode). The orange-colored, microcrystalline precipitate (II) which formed was filtered and washed with water. After drying (100°), the product weighed 23 g. (yield 71%), m. p. 216–218°. After recrystallization from acetone–alcohol–water, the melting point was 218–219° On heating above 220°, this nitro compound resolidified and gradually decomposed with sublimation of some *p*-nitrobenzoic acid. No second melting point was noticed. A sample of N^1-*p*-nitrobenzoylsulfanilamide[11] prepared from N^1-*p*-nitrobenzoyl-N^4-acetylsulfanilamide[5] showed nearly the same melting point (217°) and the same properties. A mixed melting point of the two substances showed no depression.

From the filtrate of II, *p*-nitrobenzoic acid can be recovered by precipitation with hydrochloric acid.

N^4-*p*-Nitrobenzoyl-sulfanilamide (III).—This was prepared by condensing 0.1 mole of sulfanilamide with 0.1 mole of *p*-nitrobenzoyl chloride in pyridine solution, following essentially the same procedure as described for sulfonamide I.

Summary

The *p*-nitrobenzoyl and the 3,5-di-nitrobenzoyl derivatives of a number of aromatic and hydroaromatic hydroxy and amino compounds were prepared and tested for chemotherapeutic activity in mice. The *p*-nitrobenzoyl esters of 4-hexylresorcinol showed some activity against pneumococci. In the sulfonamide series, the dibenzoyl- and the di-*p*-nitrobenzoyl-sulfanilamide were prepared as intermediates in the synthesis of N^1-benzoyl-sulfanilamide and of N^1-*p*-nitrobenzoyl-sulfanilamide. The N^1-benzoyl compound proved to be active against pneumococci while the N^1-*p*-nitrobenzoyl compound showed activity against meningococci.

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(11) This sample was kindly supplied by Dr. M. L. Crossley of the Calco Chemical Co.