4 Poser, H.; Weuffen, W.; Jülich, W.-D.: Z. Med. Labortechnik **5**, 293 (1964)
5 Spicher, G.; Peters, J.: Zbl. Bakt. Hyg., I. Abt. Orig. A **231**, 541 (1975)
6 Bundesgesundheitsbl. **85**, 397 (1982)
7 BMBF-Vorhaben 03100237 A 5: Synthese und Verwendung neuer Sulfobetaine auf der Basis nachwachsender Rohstoffe. Abschlußbericht (1995)
8 Kramer, A.; Adrian, V.; Adam, C.: Hyg. Med. **18**, 9 (1993)
9 Jülich, W.-D.; von Woedtke, Th.; Abel, P.: Deutsches Patent DE 197 09 649 A1 (1997)
10 Kühl, H.; Kramer, A.; Moll, E.: Hyg. Med. **19**, 190 (1994)
11 Positionspapier der Deutschen Gesellschaft für chemisches Apparatewesen, Chemische Technik und Biotechnologie e.V. (DECHEMA) im Auftrag des BMBF, Frankfurt/Main 1994
12 Jülich, W.-D.; Seibt, H.; Pietsch, H.; Knieler, R.; Ohme, R.; Ballschuh, D.; Kramer, A.: Deutsches Patent angemeldet am 4. 3. 1998
13 Ohme, R.; Ballschuh, D.; Seibt, H.; Jülich, W.-D.; Kramer, A.: Deutsches Patent, Patent-Nr. 1007 D 207/08, 2. 10. (1995)
14 Adrian, V.; Jülich, W.-D.; Kramer, A.; Alhitari, N.; von Woedtke, Th.; Abel, P.: Hyg. Med. **23** Suppl. 2, 94 (1998)
15 Jülich, W.-D.; Hanschke, R.; Alhitari, N.: Hyg. Med. **23**, 465 (1998)
16 Rebrin, K.; Fischer, U.; Hahn von Dorsche, H.; von Woedtke, Th.; Abel, P.; Brunstein, E.: J. Biomed. Eng. **14**, 33 (1992)
17 Urban, G.; Schlosser, M.; Jülich, W.-D.; von Woedtke, Th.; Krowas, D.; Lange, J.; Ziegler, B.: Hyg. Med. **23**, 459 (1998)
18 Jülich, W.-D.; Kindel, E.; von Woedtke, Th.; Conrads, J. P. F.: Deutsches Patent Reg.-Nr. 19748099.3 (1997)
19 Jülich, W.-D.; von Woedtke, Th.; Kindel, E.; Conrads, J. P. F.; Pfeiffer, M.: Hyg. Med. **23**, 338–344 (1998)
20 von Woedtke, Th.; Jülich, W.-D.; Kramer, A.; Nieber, D.; Pfeiffer, M.; Abel, P.; Fischer, U.: Hyg. Med. **19**, 646 (1994)
21 Jülich, W.-D.; von Woedtke, Th.: Zentr. Steril. **4**, 283 (1996)
22 von Woedtke, Th.; Jülich, W.-D.; Abel, P.; Kramer, A.: Hyg. Med. **23**, Suppl. 2, 91 (1998)
23 von Woedtke, Th.; Jülich, W.-D.; Abel, P.; Kindel, E.; Schröder, L.-W.: Hyg. Med. **23**, 346 (1998)

Eingegangen am 2. Juni 1998
Angenommen am 8. Juli 1998

Dr. W.-D. Jülich
Landeshygieneinstitut
Mecklenburg-Vorpommern
Lange Reihe 2
D-17489 Greifswald

Chemistry Department, Faculty of Science, Girl's Branch, Al-Azhar University, Nasr City, Cairo, A. R. Egypt

Synthesis and reaction of cyanopyridone derivatives and their potential biological activities

A. S. S. SALMAN

4-Carboxy-3-cyano-6-biphenyl-pyrid-2-one (**1**) was prepared and then allowed to react with methyliodide, benzenesulphonyl chloride, phenylisothiocyanate, acetic anhydride, *o*-phenylenediamine, phenylmagnesium bromide and phosphorus pentasulphide affording the corresponding N-substituted pyrid-2-one **2**–**5**, 4-(benzimidazol-2-yl)-pyrid-2-one **7**, 2-hydroxy-2-phenyl-1,2-dihydro-pyridine **8** and 2-thiopyridone **9** derivatives, respectively. Treatment of **1** with dimethyl sulphate and phosphorus oxychloride to give 2-methoxy pyridine **12** and 2-chloro pyridine **13** derivatives. Reaction of **13** with amines and hydrazine hydrate afforded **14** and **15**, respectively. The structural assignments of the new compounds were based on analytical, spectroscopic measurements and chemical reactions. Some of the obtained compounds showed interesting antibacterial and antifungal activities in vitro.

1. Introduction

In this work, the chemical activity of 4-carboxy-3-cyano-6-biphenyl-pyrid-2-one was studied to obtain several substituted pyridines for biological evaluation.

2. Investigations, results and discussion

2.1. Chemistry

Extensive studies [1, 2] described the reaction of active methylene compounds with α,β-unsaturated ketones in the presence of ammonium acetate to afford cyanopyridines through a Michael type addition, followed by cyclization. The obtained cyanopyridines were found to possess a pronounced antimicrobial activity [3, 4].

In the present work, 4-carboxy-3-cyano-6-biphenyl-pyrid-2-one (**1**) was obtained by condensation of β-(*p*-phenyl)benzoylacrylic acid with cyanoacetamide in boiling ethanol in the presence of piperidine as a catalyst. The introduction of aromatic residues into the terminal positions of the conjugate system. $\rangle C{=}\overset{|}{C}{-}C{=}O$ appears to increase its polar character and therefore its tendency to undergo the Michael condensation as well as the effect of

Scheme 1

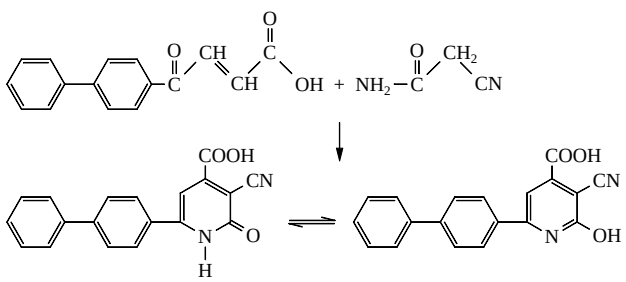


Table 1: Spectral data of compounds 1–17

Comp.	IR (cm^{-1})	^1H NMR (δ ppm)
1	1730 (C=O) carboxyl, 1675 (C=O) ring amide, 2230 (CN), 2890 (OH), 3157 (NH), 1635 (C=N),	6.7 (s, 1 H, CH heterocycl), 8.1 (s, 1 H, NH) 7.1–7.7 (m, 9 H), aromatic protons)
2	3410 (OH), 1675 (C=O), 1592 (CN), 2921 (−CH), 1725 (C=O)	2.4 (s, 3 H, N−CH_3), 6.3 (s, 1 H, CH heterocycl), 7.3–7.9 (m, 9 H, aromatic protons)
3	1670 (C=O) ring amide, 1725 (C=O carboxyl), 3330 (OH), 2225 (CN), 1391, 1332, 1188 all to (SO_2)	7.1–7.9 (m, 14 H, aromatic protons), 6.4 (s, 1 H, CH heterocycl)
4	3210 (NH), 1240 (C=S), 1592 (C−N)	8.5 (s, 1 H, NH−C(=S)−), 7.2–8.1 (m, 14 H, aromatic protons) 6.3 (s, 1 H, CH heterocycl)
5	1725 (C=O acetyl), 1718 (C=O carboxyl), 1675 (C=O ring amide), 2230 (CN), 3330 (OH)	2.4 (s, 3 H, CO−CH_3), 6.2 (s, 1 H, CH heterocycl), 7.3–7.9 (m, 9 H, aromatic protons)
6	1620 (C=N), 2225 (CN), 1716 (C=O carboxyl), 3320 (OH)	2.5 (s, 3 H, CH_3), 7.1–7.9 (m, 9 H, aromatic protons), 6.5 (s, 1 H, CH heterocycl)
7	1625 (C=N), 3157 (N−H), 1677 (C=O ring amide), 2220 (CN), 3325 (NH)	8.1 (s, 1 H, NH), 6.7 (s, 1 H, CH heterocycl), 7.3–7.9 (m, 13 H, aromatic protons), 8.5 (s, 1 H, NH)
8	2210 (CN), 3270 (NH), 1718 (C=O carboxyl), 3420 (OH)	5.9 (s, 1 H, CH heterocycl), 8.1 (s, 1 H, NH), 7.3–7.9 (m, 14 H, aromatic protons)
9	1180 (C=S), 1615 (C=N), 3160 (NH), 2820 (SH), 3010 (OH), 1750 (C=O)	9.6 (br, 1 H, SH), 10.2 (br, 1 H, NH−C=S), 7.6–8.5 (m, 9 H, aromatic protons), 6.5 (s, 1 H, heterocycl)
10	3300 (NH), 1615 (C=N), 1690 (C=O carboxyl), 2225 (CN)	10.9 (br, 1 H, NH), 6.3 (s, 1 H, CH heterocycl), 7.5–8.1 (m, 14 H, aromatic protons)
11	1620 (C=N), 1390 (C−S), 2230 (CN)	2.9–3.1 (t, 2 H, $-CH_2-CH_2-CN$), 4.1–4.3 (t, 2 H, $-S-CH_2-CH_2-$), 7.1–7.3 (m, 9 H, aromatic protons), 6.3 (s, 1 H, CH heterocycl)
12	1100 (−C−O−), 2220 (CN), 1630 (C=N), 1725 (C=O), 3031 (OH)	4.6 (s, 3 H, OCH_3), 7.1–7.5 (m, 9 H, aromatic protons), 6.5 (s, 1 H, CH heterocycl)
13	1620 (C=N), 2220 (CN), 1725 (C=O carboxyl), 3010 (OH), 1670 (C−Cl)	7.3–7.9 (m, 9 H, aromatic protons), 6.1 (s, 1 H, CH heterocycl)
14a	1625 (C=N), 3330 (NH), 1725 (C=O carboxyl), 3010 (OH)	
14b	1625 (C=N), 3290 (NH), 1310 (OH)	10.1 (s, br, 1 H, NH), 6.1 (s, 1 H, −CH heterocycl), 7.1–7.3 (m, 13 H, aromatic protons), 2.5 (s, 3 H, OCH_3)
14c	1630 (C=N), 3320 (NH), 1720 (C=O carboxyl), 3100 (OH), 2225 (CN)	
15	3400, 3180 (NH_2, NH), 1610 (C=N), 1725 (C=O carboxyl), 3102 (OH), 2215 (CN)	4.5 (s, 2 H, NH_2), 8.00 (s, 1 H, NH), 6.1 (s, 1 H, CH heterocycl), 7.1–7.5 (m, 9H, aromatic protons)
16a	2230 (CN), 3320 (NH), 1615 (C=N), 1720 (C=O carboxyl), 3200 (OH)	10.2 (s, 1 H, NH), 7.1–8.0 (m, 14 H, aromatic protons), 8.2 (s, 1 H, CH=N), 6.5 (s, 1 H, CH heterocycl)
16b	1620 (C=N), 3210 (NH), 1720 (C=O carboxyl), 3200 (OH)	7.5–8.1 (m, 13 H, aromatic protons), 8.1 (s, 1 H, CH=N), 6.3 (s, 1 H, CH heterocycl), 2.1 (s, 3 H, OCH_3)
17	3300 (NH), 1615 (C=N), 1690 (C=O)	5.4 (br, 2 H, NH−NH), 4.6 (s, 2 H, CH_2), 6.3 (s, 1 H, CH heterocycl), 3.4 (t, 3 H, $CH_2-\underline{CH_3}$), 4.1 (q, 2 H, $\underline{CH_2}-CH_3$), 7.2–7.9 (m, 9H, aromatic protons)

the presence of a carboxylic group on the polarity of the molecule [1] (Scheme 1). The structure of **1** was confirmed by elemental analysis and spectral data.

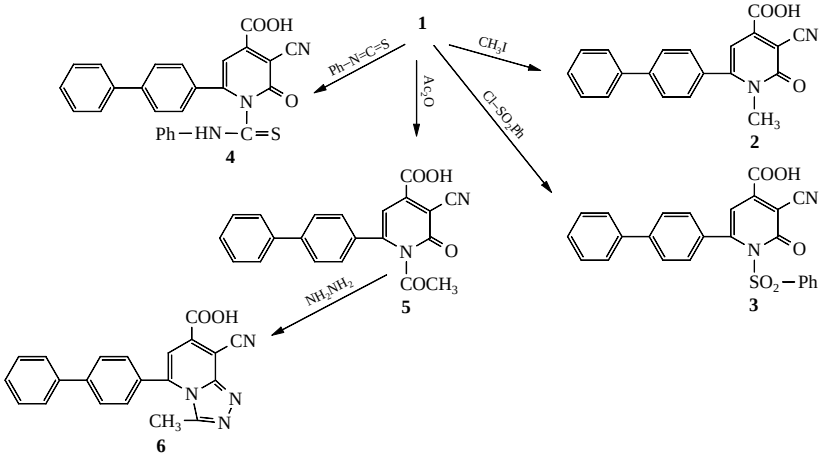
The cyanopyridone derivative **1** was used as a key intermediate in the synthesis of the N-substituted 4-carboxy-3-cyano-6-biphenyl-pyrid-2-ones **2**–**5** via the condensation with methyl iodide [5], benzene sulphonyl chloride, phenylisothiocyanate and acetic anhydride in alkaline media. The structure of new compounds were established for the reaction product based on analytical and spectral data (Table 1).

Compound **5** reacted with hydrazine hydrate in boiling ethanol to afford the corresponding triazol derivate **6** (Scheme 2).

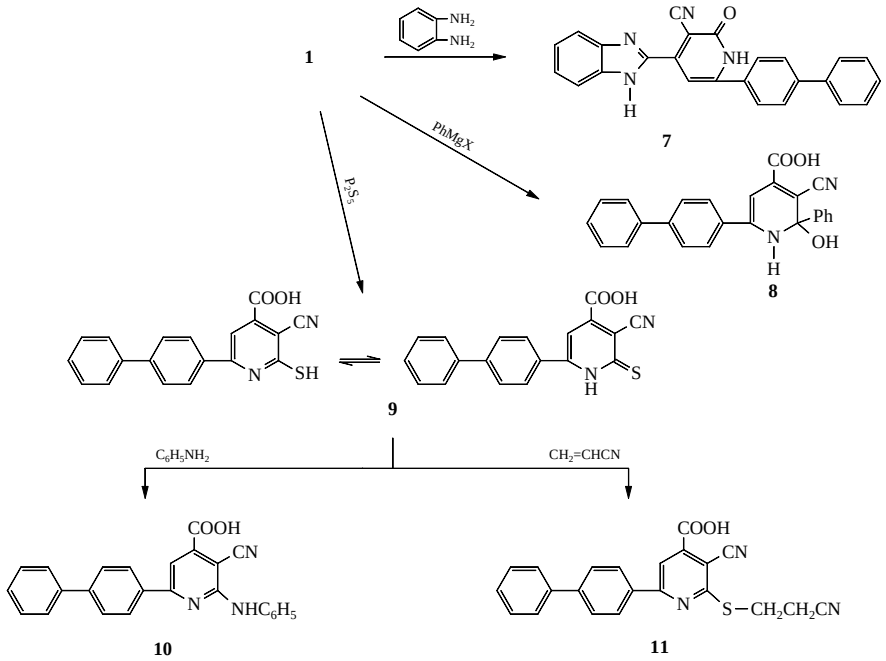
The pyrid-2-one derivative **1** proved to be an useful precursor for the synthesis of other heterocycles for biological evaluation (Table 2). Thus the reaction of this pyridone derivative **1** with *o*-phenylenediamine afforded the corresponding 4-(benzimidazol-2-yl)-3-cyanopyrid-2-one derivative **7**, the IR spectra showed absorption bands at 3220 cm^{-1} (NH), 1625 cm^{-1} C=N and the disappearance of absorption bands at 3400 cm^{-1} (OH) and 1605 cm^{-1} (C=O carboxyl).

Treatment of **1** with phenylmagnesium bromide afforded 4-carboxy-3-cyano-2-hydroxy-2-phenyl-6-biphenyl-1,2-dihydro-pyridine (**8**), IR spectra showed an absorption band at 3420 cm^{-1} (OH) and a disappearance of the absorption band at 1675 (C=O ring amid). Heating of **1** with phos-

Scheme 2



Scheme 3



phorus pentasulfide [6] in dry pyridine afforded the corresponding thiopyridine derivative **9**, IR spectra shows a broad absorption band at 3160–2820 cm^{-1}, characteristic of a $NH-\underset{\underset{S}{\|}}{C}\rightleftharpoons N=\underset{\underset{SH}{|}}{C}-$ system. The thione **9** was condensed with aniline to give the corresponding 2-anilinopyridine derivative **10**. The structures of compound **10** was also confirmed by analytical data and spectral data. The IR absorption spectra revealed an absorption band at 3220–3240 cm^{-1} (NH) and the disappearance of the absorption band at 1240 cm^{-1} (C=S). Reaction of **9** with acrylonitrile [7] in aqueous sodium hydroxide gave the 2-(2′-cyanoethylthio)-pyridine derivative **11** (Scheme 3).

Reaction of **1** with dimethyl suphate [7] in boiling dry acetone containing anhydrous potassium carbonate afforded the corresponding 2-methoxypyridine derivative **12**. The IR spectrum revealed absorption bands at 1100 cm^{-1} (C–O), 2220 cm^{-1} (C=N) and the disappearance of absorption bands at 1675 cm^{-1} (C=O) and 3300 cm^{-1} (NH).

The chloro derivative **13** was obtained by treatment of **1** with phosphorus oxychloride in the presence of phosphorus pentachloride [8, 9], which underwent nucleophilic substitution with amines and hydrazine resulting in 2-amino-/and hydrazino-4-carboxy-3-cyano-6-biphenyl-pyridines **14a–c** and **15**, respectively. The structure of **15** was further supported by its reaction with aromatic aldehydes [6] which afforded the corresponding arylidenehydrazonyl derivatives **16a**, **b**, while condensation of **15** with ethyl chloroacetate afforded the corresponding **17** (Scheme 4, Table 1).

2.2. Biological activity

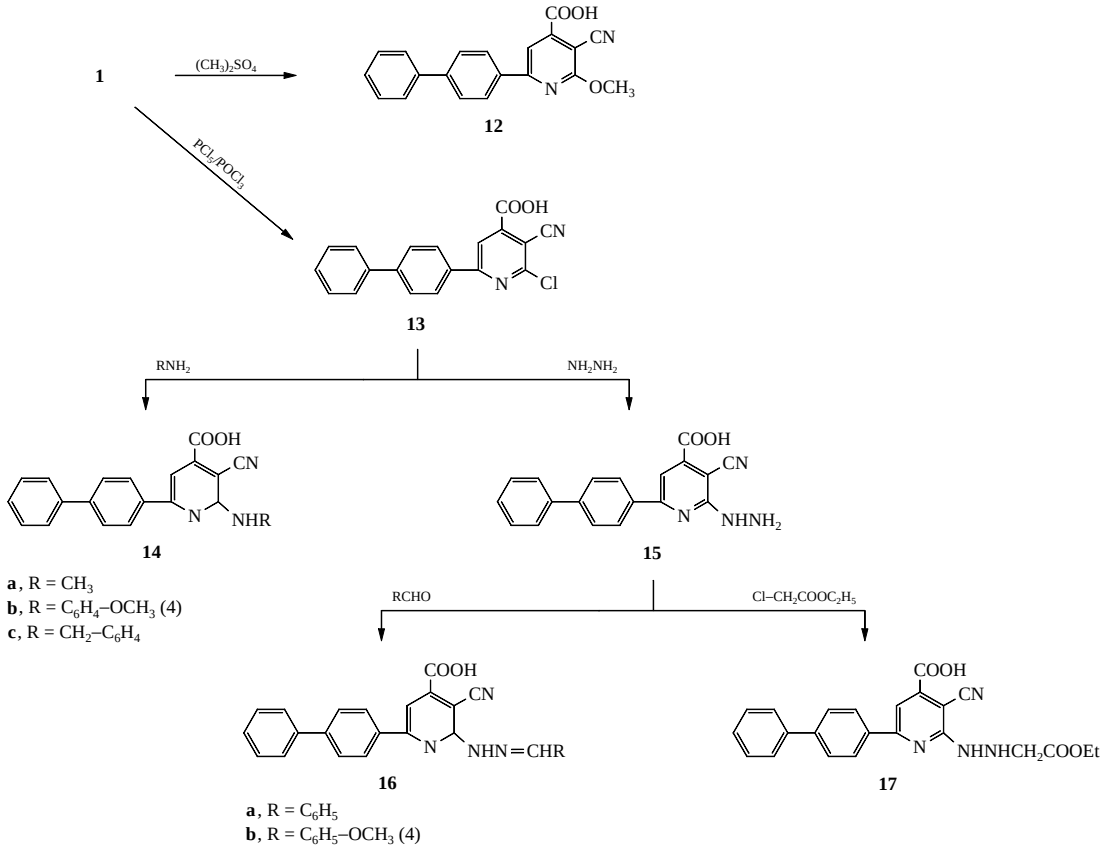
Most of the prepared compounds were tested *in vitro* for antibacterial activity (minimum inhibition concentration, MIC) against Gram-negative bacteria (*Serratia marcescens* and *Proteus mirabilis*) and Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus cereus*) using the nutrient agar pour plate method [10] at the 125 μg/ml level against the micro-organisms used. The antifungal activity

Table 2: Antibacterial and fungicidal activity of the tested compounds

Compd.	*Staphylococcus aureus* (ATCC-6538-P)	*Bacillus cereus* (NRRL-B-569)	*Serratia marcescens* (IMRU-70)	*Proteus mirabilis* (NTC-289)	*Aspergillus fumigatus* (PP-29)
1	+	+	+	+	−
3	+	+	+	+	−
4	++	+	++	+	+
5	+	+	+	+	+
6	+	+	+	+	+
7	++	++	++	++	+
9	++	++	++	++	+
13	++	++	+	+	+
14a	+	++	+	++	+
14b	++	++	++	++	−
15	++	+	++	+	+
16a	++	++	++	++	+
16b	+++	++	++	+	+
17	+++	+++	++	++	+
Amp.	++	++	++	++	−

Diameter of the zone of inhibition:
−: <1 cm; +: 1 to 1.5 cm; ++: 1.5 cm to 2 cm; +++: >2 cm. The solvent was DMF Amp.: Ampicillin

Scheme 4



of the same compounds were tested to determine the MIC using turbidimetric method [11]. The reference antibiotic ampicillin was used to evaluate the potency of the tested compounds. The test results (Table 2) revealed that compound **17** possesses the highest activity against *Staphylococcus aureus* and *Bacillus cereus*. Also compound **16b** possesses high activity against *Staphylococcus aureus* compared to ampicillin. All the tested compounds except **1**, **3** and **14b** showed mild activity against the fungus *Aspergillus fumigatus*.

3. Experimental

3.1. Apparatus and methods

All m.p's are uncorrected, IR spectra (cm^{-1}) were recorded on a Pye-Unicam spectrophotometer type 1200 using KBr Wafer technique. ^1H NMR

spectra were recorded on a Varian EM-390 (90 MHz) spectrometer using TMS as an internal standard and DMSO-d_6 as solvent. Chemical shifts were expressed in δ (ppm) values. Elemental analysis were determined using parkin-Elmer 240 C Microanalyser; all the results were in an acceptable range.

3.2. 4-Carboxy-3-cyano-6-biphenyl-pyrid-2-one (1)

To a mixture of β-(*p*-phenyl) benzoyl acrylic acid (0.01 mol) and cyanoacetamide (0.02 mol) in C_2H_5OH (50 ml) a few drops of piperidine were added; the mixture was heated under reflux for 6 h. The reaction mixture was concentrated under reduced pressure and acidified with CH_3COOH. The separated solid was filtered off, washed successively with H_2O and C_2H_5OH and crystallized from C_2H_5OH to give yellow crystals of **1**, m.p.: 240–242 °C, yield 80%.
$C_{19}H_{12}N_2O_3$ (316.3)

3.3. N-Methyl-4-carboxy-3-cyano-6-biphenyl-pyrid-2-one (2)

To a solution of **1** (0.05 mol) in pyridine (15 ml) was added CH_3I (0.05 mol). The reaction mixture was refluxed for 4 h, cooled, poured into ice-cold H_2O. The solid products separated were filtered off and crystallized from C_2H_5OH to give brown powder of **2**, m.p.: 170–173 °C, yield: 50%.
$C_{20}H_{14}N_2O_3$ (330.3)

3.4. N-Phenylsulphonyl-4-carboxy-3-cyano-6-biphenyl-pyrid-2-one (3)

To a solution of **1** (0.05 mol) in pyridine (15 ml) benzenesulphonyl chloride (0.05 mol) was added. The reaction mixture was refluxed for 4 h, cooled, poured into ice-cold H_2O. The solid products that separated was filtered off and crystallized from C_2H_5OH to give deep brown powder of **3**, m.p.: 150–154 °C; yield: 50%.
$C_{25}H_{16}N_2O_5S$ (456.5)

3.5. N-Phenylthiocarbamoyl-4-carboxy-3-cyano-6-biphenyl-pyrid-2-one (4)

To a solution of **1** (0.01 mol) in aqueous NaOH (8%), phenylisothiocyanata (0.01 mol) was added. The reaction mixture was refluxed for 4 h, cooled, poured into ice-cold H_2O. The solid products separated were filtered off and crystallized from petroleum ether (60–80 °C) to afford **4** as yellow crystals, m.p.: 110–112 °C; yield: 85%.
$C_{26}H_{17}N_3O_3S$ (451.5)

3.6 N-Acetyl-4-carboxy-3-cyano-6-biphenyl-pyrid-2-one (5)

To a solution of **1** (0.01 mol) in Ac_2O (30 mol), AcONa (1 g) was added. The reaction mixture was heated under reflux for 4 h and poured into ice-cold H_2O. The separated product was filtered off and crystallized from C_2H_5OH as deep brown powder, m.p.: 195–198 °C; yield: 65%.
$C_{21}H_{14}N_2O_4$ (358.4)

3.7. 5-Carboxy-4-cyano-1-methyl-7-biphenyl-1,2,4-triazolo[3,4-a]pyridine (6)

A mixture of **5** (0.01 mol) and hydrazine hydrate (0.01 mol) in C_2H_5OH (20 ml) was refluxed for 6 h, then allowed to cool. The solid product was collected and crystallized from C_2H_5OH as pale yellow crystals, m.p.: 160–163 °C; yield: 70%.
$C_{21}H_{14}N_4O_2$ (354.4)

3.8. 4-(Benzimidazol-2-yl)-3-cyano-6-biphenyl-pyrid-2-one (7)

A mixture of **1** (0.01 mol) and *o*-phenylenediamine (0.01 mol) in C_2H_5OH (20 ml) was refluxed for 48 h, then allowed to cool. The solid product was collected and crystallized from C_2H_5OH as dark brown powder, m.p.: 210–213 °C; yield: 60%.
$C_{25}H_{16}N_4O$ (388.3)

3.9. 4-Carboxy-3-cyano-2-hydroxy-2-phenyl-6-biphenyl-1,2-dihydro-pyridine (8)

To a well stirred suspension of Mg turnings (1 g) in 30 ml of dry $(C_2H_5)_2O$/benzene mixture (1:1), bromobenzene (0.01 mol) in 30 ml of dry $(C_2H_5)_2O$/bezene was added and stirring was continued at 30 °C for 30 min. A suspension of **1** (0.01 mol) in 30 ml of a dry $(C_2H_5)_2O$/benzene mixture was added dropwise add and stirring was continued for 1 h. Then the reaction mixture was heated on a steam – bath for 5 h and left overnight. It was decomposed with a saturated solution of NH_4Cl. The organic layer was extracted and washed well. The etheral solution was dried and then evaporated under reduced pressure. The solid was collected and crystallized from benzene as brown crystals, m.p.: 130–133 °C; yield: 60%.
$C_{25}H_{18}N_2O_3$ (394.3)

3.10. 4-Carboxy-3-cyano-6-biphenyl-2-thiopyridone (9)

To a solution of cyanopyridone **1** (0.01 mol) in dry pyridine (20 ml), P_2S_5 (0.02 mol) was added, and the reaction mixture was refluxed for 6 h. Then it was cooled and poured into an ice/H_2O mixture. The separated solid was filtered off, washed with H_2O, dried and crystallized from benzene to give a dark brown powder of **9**, m.p.: 180–184 °C; yield: 60%.
$C_{19}H_{12}N_2O_2S$ (332.4)

3.11. 2-Anilino-4-carboxy-3-cyano-6-biphenyl-pyridine (10)

A mixture of **9** (0.01 mol) and aniline (3 ml) was heated at 170–180 °C for 4 h. On cooling and dilution with C_2H_5OH, yellow crystals separated. The reaction mixture was heated on a water-bath for 8 h. The solid product was collected and crystallized from benzene/petroleum ether (60–80 °C) to afford **10** as reddish brown powder; m.p.: 220–222 °C; yield: 50%.
$C_{25}H_{17}N_3O_2$ (391.4)

3.12. 2-(2-Cyanoethylthio)-4-carboxy-3-cyano-6-biphenyl-pyridine (11)

A mixture of **9** (0.01 mol) and acrylonitrile (0.01 mol) in 20 ml of pyridine was refluxed for 3 h. Then it was cooled and poured into an ice/HCl mixture. The separated solid was filtered off, washed with H_2O, dried and crystallized from ethanol to give a brown powder of **11**, m.p.: 150–153 °C; yield: 55%.
$C_{25}H_{15}N_3O_2S$ (385.4)

3.13. 2-Methoxy-4-carboxy-3-cyano-6-biphenyl-pyridine (12)

A mixture of **1** (0.01 mol), dimethyl sulphate (0.01 mol) and anh. K_2CO_3 (0.4 g) in dry acetone (50 ml) was refluxed for 24 h. Then it was cooled and poured into an ice/H_2O mixture. The separated solid was filtered off and crystallized from acetone to give yellow crystals (**12**); m.p.: 160–164 °C; yield: 45%.
$C_{20}H_{14}N_2O_3$ (330.3)

3.14. 4-Carboxy-2-chloro-3-cyano-6-biphenyl-pyridine (13)

A solution of **1** (0.01 mol) in $POCl_3$ (10 ml) was heated on a boiling water bath for 1 h in the presence of PCl_5 (1 g). The reaction mixture was cooled and poured into crushed ice. The obtained solid was separated by filteration, dried and crystallized from benzene to give **13**, m.p.: 200–202 °C; yield: 60%.
$C_{19}H_{11}ClN_2O_2$ (334.8)

3.15. 2-Amino-4-carboxy-3-cyano-6-biphenyl-pyridines 14a–c

A mixture of **13** (0.01 mol) and an appropriate amine (0.02 mol) in abs. C_2H_5OH (30 ml) was refluxed for 6–8 h. The excess of solvent was removed under reduced pressure and the residue was washed with H_2O. The crude material gave, on trituration with petroleum ether, a solid which was crystallized from C_2H_5OH to give **14a–c.**
14a (R=CH_3): yellow crystals, m.p.: 140–143 °C; yield: 55%.
$C_{20}H_{15}N_3O_2$ (329.4)
14b (R=$C_6H_4OCH_3$ (4)): brown powder, m.p.: 130–132 °C; yield: 60%.
$C_{26}H_{19}N_3O_3$ (421.5)
14c R=($CH_2-C_6H_5$): white crystals, m.p.: 155–157 °C; yield: 70%.
$C_{26}H_{19}N_3O_2$ (405.5)

3.16. 4-Carboxy-3-cyano-2-hydrazino-6-biphenyl-pyridine (15)

A mixture of **13** (0.01 mol), and hydrazine hydrate (0.01 mol) in abs. C_2H_5OH (30 ml) was refluxed for 6 h, and cooled. The solid formed was filtered off and crystallized from acetone to give white crystals of **15**, m.p.: 170–737 °C; yield: 60%.
$C_{19}H_{14}N_4O_2$ (330.3)

3.17. Arylidenehydrazonyl derivatives 16a, b

A mixture of **15** (0.01 mol) and aromatic aldehyde (0.01 mol) in abs. C_2H_5OH (30 ml) was refluxed for 3 h. The solid obtained was filtered off and crystallized from C_2H_5OH to give **16a**, **b**.
16a (R=C_6H_5): yellow crystals, m.p.: 100–102 °C; yield: 85%.
$C_{26}H_{18}N_4O_2$ (418.3)
16b (R=$C_6H_4OCH_3$ (4)): yellow crystals, m.p.: 138–140 °C; yield: 80%.
$C_{27}H_{20}N_4O_3$ (448.5)

3.18. 4-Carboxy-3-cyano-2-carbethoxymethyl hydrazino-6-biphenylpyridine (17)

A mixture of **15** (0.01 mol) and ethyl chloroacetate (0.01 mol) in 30 ml of sodium ethoxide (0.4 g sodium metal in 30 ml of C_2H_5OH), was refluxed for 6 h. After cooling the precipitated product was filtered off and crystallized from EtOH to give yellow crystals of **17**, m.p.: 120–122 °C; yield: 75%.
$C_{23}H_{20}N_4O_4$ (416.4)

References

1 Soliman, F. M.; El-Hashash, M. A.; Salman, A. S.: J Serb. Chem. Soc. **56**, 377 (1991)
2 Attia, A.; Abo-Ghalia, M.; Abdel-Salam, O.: Pharmazie **50**, 455 (1995)
3 Moussa, H. H.; Chabaka, L. M.: Egypt. J. Chem. **26**, 267 (1983)
4 Attia, A.; Michael, M.: Pharmazie **37**, 551 (1982)
5 Al-Hajar, F. H.; Sabri, S. S.: J. Heterocyclic. Chem. **19**, 1087 (1982)
6 Ram, V. J.; Pandey, H. K.; Vlietinck, A. J.: J. Heterocyclic Chem. **18**, 1277 (1981)
7 Soliman, F. M.; El-Hashash, M. A.; Sauka, L. M.; Salman, A. S.: Rev. Roum. Chem. **41**, 109 (1996)
8 Kamal-El-Dean, A. M.; El-Kasef, H. S.: Pharmazie **51**, 155 (1996)
9 Ismail, M. F.; Sayed, F. S.; El-Khamry, A. A.; Ali, M. A.; Mansour, M. M.: J. Prakt. Chem. **331**, 399 (1989)
10 Stewart, F. S.; Berwich: Bacteriology, Virology and Immunity for Students of Medicine, 10th ed., p. 68, 1979
11 Goweulock, v. A. H.; Bell, M.: Practical Chemical Biochemistry, Vol. 1, 5th ed. p. 179, 1980

Received February 28, 1998
Accepted May 15, 1998

Dr. Asmaa Said Salem Salman
Chemistry Department
Faculty of Science
Al-Azhar University, Girl's Branch
Nasr City, Cairo,
Egypt

Groningen Institute for Drug Studies (GIDS), Departments of Analytical Chemistry & Toxicology[1] and Medicinal Chemistry[2], University Centre for Pharmacy, State University of Groningen, The Netherlands, Astra Arcus[3], Preclinical R & D, Södertälje, Sweden, and Astra Hässle[4], Division of Analytical Chemistry, Mölndal, Sweden

Temperature effects on the chromatographic behaviour of racemic 2-amidotetralins on a Whelk-O 1 stationary phase in super- and subcritical fluid chromatography

U. Selditz[1], S. Copinga[3], C. J. Grol[2], J. P. Franke[1], R. A. de Zeeuw[1], H. Wikström[2] and O. Gyllenhaal[4]

The chromatographic behaviour of thirty structurally related racemic 2-amidotetralins on a *cis*-(3*R*, 4*S*)-Whelk-O 1 stationary phase was investigated at five different temperatures between 50 and −15 °C using super-or subscritical fluid chromatography (SFC/SubFC). Generally, low selectivity factors were observed for this class of analytes on this stationary phase. In particular, phenyl and benzyl substituted analogues were strongly retained, probably due to strong π−π interactions with the chiral selector molecule, but the enantioselectivity was low. This also counts for the two *p*-iodo substituted methoxy analogues, although here steric effects may be responsible for the high capacity factors. In about 10% of the cases, a decrease in the (surrounding) temperature of the column did not result in an improved selectivity. Only four compounds out of thirty could not be resolved under the given conditions.

1. Introduction

Retention in chromatography is dependent on the free energy of the partitioning process of an analyte between the mobile and the stationary phase. In order to estimate the enthalpic and entropic contributions to this analyte transfer, van't Hoff plots are usually generated, in which the natural logarithms of experimentally determined retention parameters are plotted against the reciprocal of absolute temperature at constant pressure. The slope of such a graph, which theoretically should be linear, yields the enthalpy of the analyte transfer [1, 2]. However, there have also been reports about nonlinear van't Hoff plots where deviations from the theory are explained by alterations of the adsorption or dissociation processes, or by changes in the mobile phase interactions with either the analyte or the stationary phase [2].

In the equilibrium state, the capacity factor k_i' of a certain analyte can be related via the distribution coefficient K_D to the temperature according to equations 1a and 1b, respectively, where in (a) ΔG^0 refers to the change in Gibbs free energy of the analyte transfer between the mobile and stationary phase, R is the ideal gas constant, T is the absolute temperature, and in (b) $V_{m,s}$ are the volumes of the mobile and stationary phases, respectively, $C_{s,m}$ are the respective analyte concentrations in the two phases, and φ is defined as the phase ratio.

$$\Delta G^0 = -RT \ln K_D \tag{1a}$$

$$K_D = C_s/C_m = k_i'(V_m/V_s) = k_i'/\varphi \tag{1b}$$

According to the Gibbs-Helmholtz equation (2), the change in free energy can also be expressed in enthalpic and entropic terms,

$$\Delta G^0 = \Delta H^0 - T\,\Delta S^0 \tag{2}$$

where ΔH^0 and ΔS^0 are the respective changes in enthalpy and entropy of the analyte transfer. When substituting eq. 1a with 1b and combining eqs. 1 and 2, the solvation to $\ln k_i'$ will generate the following relationship:

$$\ln k_i' = -\Delta H^0/RT + \Delta S^0/R + \ln \varphi \tag{3}$$

From this equation it can be concluded, that the enthalpic contribution to retention decreases with temperature, whereas the entropic contribution is independent of temperature. The former parameter can therefore be obtained experimentally by determining the analyte retention as a function of temperature at constant density. The slope of the $\ln k'$ vs. $1/T$ plot represents $-\Delta H^0/R$, whereas the entropy of transfer can be obtained from the y-intercept, if the phase ratio can be estimated [3]. This is, however, not necessary when we look at the selectivity factor α, which is defined as the ratio of the capacity factors of the respective enantiomers. Now, eq. 3 can be rearranged to

$$\ln \alpha = \delta\,\Delta H^0/RT + \delta\,\Delta S^0/R \tag{4}$$