

Carbonyl Thiourea 및 Carbonyl Thiosemicarbazide의 窒素置換化合物의 合成 및 抗結核作用에 關한 研究

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Nam Bok Lee: Synthesis and Antitubercular Activity of the Nitrogen Substituents of Carbonylthioureas and Carbonylthiosemicarbazides.

Abstract—Sixteen compounds of N-aryl-N'-(4'-alkoxyphenyl) thiourea derivative, twenty one compounds of N-aryl-N'-(3'-hydroxy-4'-alkoxycarbonylphenyl) thiourea derivative, three compounds of N-aryl-N'- α -naphthylthiourea derivative and three compounds of N-cyanoacetyl-N'-aryl thiosemicarbazide derivative were synthesized as potential antitubercular agent and were subjected to screening test for antitubercular activity against *Mycobacterium tuberculosis* H₃₇Rv by tube dilution method *in vitro*. Of the forty three compounds, N-*p*-cyanobenzoyl-N'-(4'-*iso*-butoxyphenyl) thiourea, N-*p*-cyanobenzoyl-N'-(4'-*n*-amoxyphenyl)thiourea, N-furoyl-N'-(4'-*n*-amoxyphenyl) thiourea, N-*p*-cyanobenzoyl-N'-(3'-hydroxy-4'-ethoxycarbonylphenyl)thiourea, N-*p*-cyanobenzoyl-N'-(3'-hydroxy-4'-*n*-butoxycarbonylphenyl)thiourea, N-*p*-cyanobenzoyl-N'-(3'-hydroxy-4'-*n*-amoxycarbonylphenyl)thiourea, N-*p*-cyanobenzoyl-N'-(3'-hydroxy-4'-*iso*-amoxycarbonylphenyl)thiourea, and N-5-nitrofuroyl-N'-(3'-hydroxy-4'-*n*-amoxycarbonylphenyl)thiourea showed the growth inhibition of *M. tuberculosis* H₃₇Rv at the concentration of 10 μ g/ml, respectively.

1941年 Mayer等¹⁾은 抗結核作用과 抗黴作用間에는 相關關係가 成立된다는 假說을 定立하고 硫黃化合物에 對하여 研究하던中 thiourea가 抗結核作用을 나타낸다는 事實을 最初로 發表하였다. 後 1946年 Jouin과 Buu-Hoi²⁾는 thiourea 및 thiourea 誘導體의 抗結核作用을 廣範圍하게 試驗한 結果를 報告하였다. 한便 Block³⁾과 Ishidate等⁴⁾은 各各 *p*-aminophenyl

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alkylether 類가 結核菌의 發育을 阻止한다는 事實을 報告하였다.

1953年 Mayer等⁵⁾은 *p*-aminophenylalkyl ether의 thiourea 誘導體 350種에 對한 抗結核作用을 檢討한 結果 4,4'-diethoxythiocarbaniide가 結核菌을 感染시킨 實驗動物에 對하여 強力한 抗菌力을 나타낼뿐만아니라 既存 抗結核劑인 streptomycin 耐性菌株에 對하여 交叉耐性을 發現하지 않는다고 報告하였다. 그後 많은 새로운 thiourea 誘導體가 여러研究者⁶⁻²²⁾에 依하여 合成되어 *in vitro* 및 *in vivo*에서의 抗結核作用試驗結果가 報告되었는데 *p*-位에 alkoxy group를 가진 thiocarbaniide에서 比較的 높은 抗菌力을 보여주고있음은 興味로운 事實이다.

現在 臨床적으로 結核 및 癩病治療目的으로 使用되는 thiourea 誘導體는 *p,p'*-diisoamoxythiocarbaniide,²³⁻²⁵⁾ *p,p'*-diethoxythiocarbaniide²⁶⁻²⁹⁾ 및 *p*-butoxy-*p'*-dimethylaminothiocarbaniide^{30,31)} 等이다.

前述한 thiourea 誘導體는 thiourea linkage를 가진 化合物이었으나, 1956年 Murav'eva³²⁾ 等은 carbonylthiourea linkage를 가진 새로운 誘導體를合成하여 抗結核試驗結果를 發表하였다.

Bednraz³³⁾는 *p*-nitrobenzoylthiourea 誘導體 및 *p*-nitrobenzoylthiosemicarbazide 誘導體를 合成하여 抗菌試驗結果를 報告하였으며, Lipp等³⁴⁾은 acyl, aroyl 및 heterocyclic compounds의 isonicotinylthiosemicarbazide 52種을 抗結核劑로서 合成하였다.

Carbonylthiourea 系化合物中에서는 N₁-benzoyl-N₂-thiourea³⁵⁾가 結核補助治療劑로서 臨床적으로 試用되고 있다.

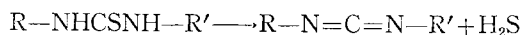
一次結核治療劑의 하나인 PAS (*p*-aminosalicylic acid)가 強力한 抗結核作用을 發現한다는 事實이 Lehmann³⁶⁾에 依하여 最初로 報告된 以來 PAS의 大量服用의 不便, 胃腸障害 및 長期服用으로 因한 PAS 耐性菌株出現等の 缺點을 除去하려는 方向으로 研究가 進行되어 왔다.

即 最近 Fujikawa等³⁷⁾은 PAS의 thiourea 誘導體를 合成하여 *in vitro*에서 試驗한 結果 PAS 感受性菌株과 PAS 耐性菌株에 對하여 거의 同一한 抗結核作用을 나타낸다는 注目할만한 事實을 發表하였다.

이事實은 PAS의 thiourea 誘導體가 PAS와 交叉耐性을 發現할수 없는 것으로 考察되기 때문에 PAS 耐性菌株 出現抑制를 爲한 새로운 合成品의 開發을 示唆하였다.

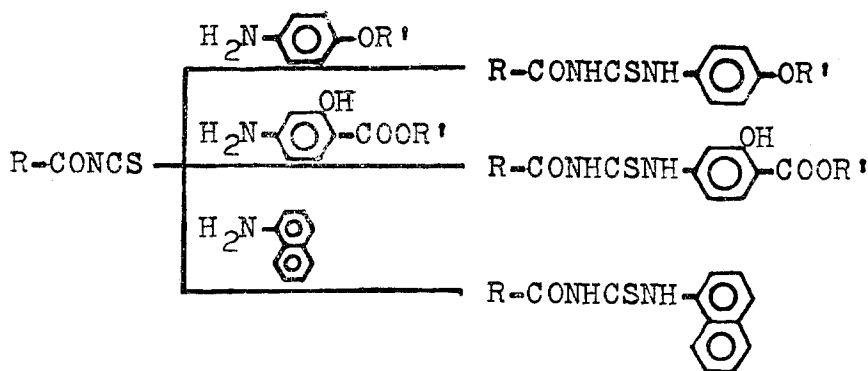
Thiourea 誘導體의 抗結核作用 mechanism은 Dieke³⁸⁾, Nestler,³⁹⁾ Smith^{40,41)} 및 Seydel等⁴²⁾에 依하여 다음과 같이 說明되고 있다.

即 thiourea 構造는 作用運搬體(action carrier)의 役割을 하며 生體內에서 이 構造는 enol 化가 抑制되어 여기에서 分離된 H₂S가 抗結核作用을 發現하는 것으로 說明된다.

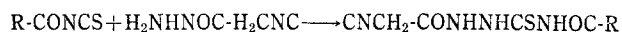


著者는 PAS를 爲始한 既存抗結核劑와 交叉耐性을 發現하지 않는 새로운 抗結核化學療法

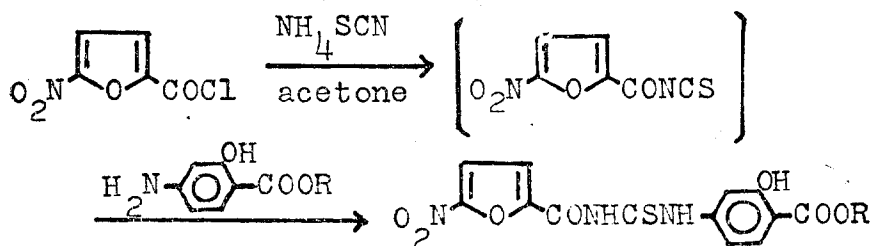
劑를 合成할 目的으로 抗結核作用이 比較的優秀한 *p*-alkoxyaniline, PAS의 alkyl ester, cyanoacetic acid hydrazide 및 α -naphthylthiourea 誘導體等化合物에 現在까지 antitubercular agent의 action moiety로 알려진 thiureido moiety를 carbonyl thiureido moiety로 代替하고 抗菌劑의 主要 residue를 이루는 phenyl, *p*-cyanophenyl, furyl 및 5-nitrofuryl group를 導入한 carbonylthiourea 誘導體 및 carbonyl thiosemicarbazide 誘導體 43種을 Scheme 1~3과 같이 合成하여 人型結核菌 H₃₇Rv에 對한 抗菌力試驗을 遂行하였기 그 結果를 報告코저 한다.



Scheme 1—Synthesis of N-aryl-N'-arylthiourea.



Scheme 2—Synthesis of N-cyanoacetyl-N'-arylthiosemicarbazide.




















Scheme 3—Synthesis of N-5-nitrofuryl-N'-(3'-hydroxy-4'-alkoxycarbonylphenyl)thiourea.

實驗 吳 結果

合 成

p-Cyanobenzoyl isothiocyanate의 合成—*p*-Cyanobenzoyl chloride 41.4g (0.25 mole)과 Pb(SCN)₂ 43.5g(0.15 mole)을 benzene 200ml에 넣고 水浴에서 3時間 還流한 後 室溫으

Table I—N-Aroyl-N'-(4'-alkoxyphenyl) thiourea. R-CONHCSNH--OR'

Compd. No.	R	R'	Formula	Recryst.* solvent	Appearance	Yield (%)	mp	Analysis(%)	
								Calcd.	Found
1.		CH ₃	C ₁₅ H ₁₄ O ₂ N ₂ S	E	colorless needles	91.3	152—153	C 62.92 H 4.93 N 9.78	62.57 4.84 9.84
2.		<i>n</i> -C ₃ H ₇	C ₁₇ H ₁₈ O ₂ N ₂ S	A	pale yellow needles	89.8	128—130	C 64.94 H 5.77 N 8.91	64.75 5.81 8.76
3.		<i>n</i> -C ₄ H ₉	C ₁₈ H ₂₀ O ₂ N ₂ S	E	colorless needles	87.4	156—157	C 65.83 H 6.14 N 8.53	65.72 6.09 8.31
4.		<i>iso</i> -C ₄ H ₉	C ₁₈ H ₂₀ O ₂ N ₂ S	E	colorless needles	82.7	140—142	C 65.83 H 6.14 N 8.53	65.53 6.21 8.76
5.		<i>n</i> -C ₅ H ₁₁	C ₁₉ H ₂₂ O ₂ N ₂ S	A	colorless needles	84.3	127—128	C 66.64 H 6.48 N 8.18	66.78 6.52 8.16
6.		<i>iso</i> -C ₅ H ₁₁	C ₁₉ H ₂₂ O ₂ N ₂ S	A	colorless needles	86.6	135—138	C 66.64 H 6.48 N 8.18	66.39 6.51 8.36
7.	NC- 	C ₂ H ₅	C ₁₇ H ₁₅ O ₂ N ₃ S	E	yellow prism	78.2	162—164	C 62.75 H 4.65 N 12.91	62.58 4.63 12.87
8.	NC- 	<i>iso</i> -C ₃ H ₇	C ₁₈ H ₁₇ O ₂ N ₃ S	E	yellow prism	82.1	158—159	C 63.70 H 5.05 N 12.38	63.22 5.02 12.33
9.	NC- 	<i>iso</i> -C ₄ H ₉	C ₁₉ H ₁₉ O ₂ N ₃ S	A	pale yellow prism	77.6	142—145	C 64.57 H 5.42 N 11.89	64.29 5.47 11.81
10.	NC- 	<i>n</i> -C ₅ H ₁₁	C ₂₀ H ₂₁ O ₂ N ₃ S	E	yellow prism	78.4	123—125	C 65.37 H 5.76 N 11.43	65.18 5.81 11.25
11.		C ₂ H ₅	C ₁₄ H ₁₄ O ₃ N ₂ S	E	colorless needle	93.5	108—111	C 57.92 H 4.86 N 9.65	57.74 4.59 9.79
12.		<i>n</i> -C ₃ H ₇	C ₁₅ H ₁₆ O ₃ N ₂ S	E	colorless needle	91.8	122—125	C 59.19 H 5.30 N 9.20	59.51 5.41 9.34
13.		<i>n</i> -C ₄ H ₉	C ₁₆ H ₁₈ O ₃ N ₂ S	E	colorless needle	89.5	115—116	C 60.35 H 5.70 N 8.80	59.99 5.59 8.71
14.		<i>iso</i> -C ₄ H ₉	C ₁₆ H ₁₈ O ₃ N ₂ S	E	colorless prism	87.2	139—141	C 60.35 H 5.70 N 8.80	60.44 5.76 8.82
15.		<i>n</i> -C ₅ H ₁₁	C ₁₇ H ₂₀ O ₃ N ₂ S	E	colorless needle	82.9	99—100	C 61.42 H 6.06 N 8.43	61.81 6.12 8.28
16.		<i>iso</i> -C ₅ H ₁₁	C ₁₇ H ₂₀ O ₃ N ₂ S	E	pale yellow prism	78.3	137—140	C 61.42 H 6.06 N 8.43	61.53 6.03 8.51

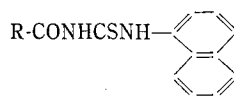
* E; EtOH, A; (Me)₂CO




Table II—N-Aroyl-N'-(3'-hydroxy-4'-alkoxycarbonylphenyl) thiourea.

$$\text{R}-\text{CONHCSNH}-\text{C}_6\text{H}_2(\text{OH})(\text{COOR}')$$

Compd. No.	R	R'	Formula	Recryst.* solvent	Appearance	Yield (%)	mp	Analysis(%)	
								Calcd.	Found
17.		CH ₃	C ₁₆ H ₁₄ O ₄ N ₂ S	A	colorless prism	84.3	201—202	C 58.17 H 4.27 N 8.48	57.94 4.31 8.42
18.		C ₂ H ₅	C ₁₇ H ₁₆ O ₄ N ₂ S	A	colorless prism	81.5	139—142	C 59.29 H 4.68 N 8.13	59.11 4.72 8.53
19.		<i>n</i> -C ₄ H ₉	C ₁₉ H ₂₀ O ₄ N ₂ S	E	colorless needle	74.5	119—120	C 61.27 H 5.41 N 7.53	61.6 5.5 7.4
20.		<i>iso</i> -C ₄ H ₉	C ₁₉ H ₂₀ O ₄ N ₂ S	E	colorless prism	72.4	129—132	C 61.27 H 5.41 N 7.53	61.32 5.31 7.57
21.	NC-	C ₂ H ₅	C ₁₈ H ₁₅ O ₄ N ₃ S	A	colorless plate	73.8	210—212	C 58.58 H 4.09 N 11.38	58.73 4.13 11.41
22.	NC-	<i>n</i> -C ₃ H ₇	C ₁₉ H ₁₇ O ₄ N ₃ S	A	pale yellow plate	69.2	206—207	C 59.52 H 4.47 N 10.96	59.87 4.20 10.94
23.	NC-	<i>n</i> -C ₄ H ₉	C ₂₀ H ₁₉ O ₄ N ₃ S	A	pale yellow plate	68.6	162—164	C 60.44 H 4.82 N 10.57	60.15 4.79 10.47
24.	NC-	<i>so</i> -C ₄ H ₉	C ₂₀ H ₁₉ O ₄ N ₃ S	70% E	yellow needle	54.3	177—179	C 60.44 H 4.82 N 10.57	60.53 4.87 10.75
5.	NC-	<i>n</i> -C ₅ H ₁₁	C ₂₁ H ₂₁ O ₄ N ₃ S	E	pale yellow cryst. powder	62.5	150—151	C 61.30 H 5.14 N 10.21	61.42 5.17 10.32
26.	NC-	<i>iso</i> -C ₅ H ₁₁	C ₂₁ H ₂₁ O ₄ N ₃ S	A	yellow prism	59.8	164—166	C 61.30 H 5.14 N 10.21	61.85 5.08 10.14
27.		CH ₃	C ₁₄ H ₁₂ O ₅ N ₂ S	A	colorless plate	84.9	197—198	C 52.50 H 3.78 N 8.75	52.73 3.71 8.94
28.		C ₂ H ₅	C ₁₅ H ₁₄ O ₅ N ₂ S	E	light brown prism	91.3	155—157	C 53.89 H 4.22 N 8.38	53.62 4.26 8.41
29.		<i>n</i> -C ₃ H ₇	C ₁₆ H ₁₆ O ₅ N ₂ S	E	pale yellow needle	89.5	152—153	C 55.16 H 4.63 N 8.04	54.98 4.58 8.12
30.		<i>iso</i> -C ₃ H ₇	C ₁₆ H ₁₆ O ₅ N ₂ S	A	colorless plate	83.2	142—143	C 55.16 H 4.63 N 8.04	55.33 4.75 8.21
31.		<i>n</i> -C ₄ H ₉	C ₁₇ H ₁₈ O ₅ N ₂ S	E	colorless plate	88.5	139—140	C 56.34 H 5.01 N 7.73	55.98 4.84 7.81
32.		<i>n</i> -C ₅ H ₁₁	C ₁₈ H ₂₀ O ₅ N ₂ S	A	light brown prism	81.7	142—143	C 57.43 H 5.36 N 7.44	57.69 5.41 7.28

* E; EtOH, A; (Me)₂CO




Table III—N-Aroyl-N'- α -naphthyl thiourea.

Compd. No.	R	Formula	Recryst.* solvent	Appearance	Yield (%)	mp	Analysis(%)	
							Calcd.	Found
33.		C ₁₈ H ₁₄ ON ₂ S	E	yellow needie	94.3	164—166	C 70.56 H 4.61 N 9.14	70.36 4.51 8.96
34.	NC- 	C ₁₉ H ₁₃ ON ₃ S	A	pale yellow cryst. powder	82.4	208—210	C 68.86 H 3.95 N 12.68	69.12 3.89 12.59
35.		C ₁₆ H ₁₂ O ₂ N ₂ S	A	colorless prism	88.7	185—186	C 64.85 H 4.08 N 9.45	65.12 4.15 9.66

E; EtOH, A; (Me)₂CO

Table IV —N-Cyanoacetyl-N'-aroyl thiosemicarbazide.



Compd. No.	R	Formula	Recryst.* solvent	Appearance	Yield (%)	mp(°C)	Analysis(%)	
							Calcd.	Found
36.		C ₁₁ H ₁₀ O ₂ N ₄ S	A	colorless cryst. powder	83.3	176	C 50.37 H 3.84 N 21.36	50.48 3.76 21.72
37.	NC- 	C ₁₂ H ₉ O ₂ N ₅ S	E	yellow prism	74.2	200—203	C 50.17 H 3.16 N 24.38	50.40 3.12 24.73
38.		C ₉ H ₈ O ₃ N ₄ S	A	pale yellow prism	76.8	188—190	C 42.85 H 3.20 N 22.21	42.39 3.19 22.14

* E; EtOH, A; (Me)₂CO

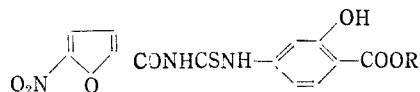
로 冷却하고 濾過하여 未反應의 Pb(SCN)₂ 와 PbCl₂ 를 除去한다. Benzene 을 溜去하고 殘留物을 dioxane 으로 再結晶하여 無色の 結晶性 粉末을 얻는다. mp 85-88°, 29.8g (63.4%)

Anal. Calcd. for C₉H₈O₃N₄S: C, 56.66; H, 2.67; N, 15.05. Found: C, 56.84; H, 2.71; N, 15.21

N-Aroyl-N'-(4'-alkoxyphenyl) Thiourea 의 合成—Aroyl isothiocyanate 0.01 mole 과 *p*-alkoxyaniline 0.01 mole 을 benzene 20ml 에 溶解하고 水浴에서 2時間 還流하여 反應시킨다. 水浴에서 acetone 을 蒸溜하고 殘留物을 acetone 또는 EtOH 로 再結晶한다 (Table I).

N-Aroyl-N'-(3'-hydroxy-4'-alkoxycarbonylphenyl) Thiourea 의 合成—Aroyl isothiocyanate 및 alkyl *p*-aminosalicylate 각 0.01 mole 을 acetone 20ml 에 溶解하고 2時間 還流하여 反應시킨다. 水浴에서 溶媒를 溜去하고 殘留物을 acetone 또는 EtOH 로 再結晶한다 (Table II).

Table V—N-5-Nitrofuoyl-N'-(3'-hydroxy-4'-alkoxy carbonylphenyl) thiourea.



Compd. No.	R	Formula	Recryst.* solvent	Appearance	Yield (%)	mp	Analysis(%)	
							Calcd	Found
39.	C ₂ H ₅	C ₁₅ H ₁₃ O ₇ N ₃ S	A	yellow cryst. powder	84.9	181—183 (Dec.)	C 47.49 H 3.45 N 11.08	47.82 3.41 11.15
40.	<i>iso</i> -C ₃ H ₇	C ₁₆ H ₁₅ O ₇ N ₃ S	A	yellow cryst. powder	76.3	181—184 (Dec.)	C 48.85 H 3.84 N 10.68	48.36 3.75 10.81
41.	<i>n</i> -C ₃ H ₉	C ₁₇ H ₁₇ O ₇ N ₃ S	E	brown cryst. powder	74.7	126—127	C 50.12 H 4.21 N 10.31	50.42 4.17 10.51
42.	<i>iso</i> -C ₄ H ₉	C ₁₇ H ₁₇ O ₇ N ₃ S	E-A	yellow cryst. powder	69.5	155—157	C 50.12 H 4.21 N 10.31	50.33 4.04 10.31
43.	<i>n</i> -C ₅ H ₁₁	C ₁₈ H ₁₉ O ₇ N ₃ S	E	yellow cryst. powder	72.4	118—119	C 51.30 H 4.54 N 9.97	51.27 4.64 10.21

* E; EtOH, A; (Me)₂CO

N-Aroyl-N'-(α -naphthyl) Thiourea 의 合成—Aroyl isothiocyanate 0.01 mole 과 α -naphthylamine 0.01 mole 을 acetone 20ml 에 溶解하고 水浴에서 2時間 還流하여 反應시킨後 反應溶媒를 溜去하고 殘留物을 acetone 또는 EtOH 로 再結晶한다(Table III).

N-Cyanoacetyl-N'-Aroyl Thiosemicarbazide 의 合成—Aroyl isothiocyanate 0.01 mole 과 cyanoacetic acid hydrazide 0.01 mole 을 acetone 에 溶解하고 水浴에서 2時間 還流하여 反應시킨後 acetone 을 溜去하고 殘留物을 acetone 또는 EtOH 로 再結晶 한다(Table IV).

N-5-Nitrofuoyl-N'-(3'-hydroxy-4'-alkoxycarbonylphenyl) Thiourea 의 合成—5-Nitrofuoyl chloride 0.005 mole 과 ammonium thiocyanate 0.006 mole 을 acetone 에 溶解하고 水浴에서 1시간 還流하여 反應시킨다. 여과하여 副生한 NH₄Cl을 除外하고 여액에 alkyl *p*-aminosalicylate 0.005 mole 을 acetone 에 溶解한 液을 合하여 水浴에서 2時間 還流시킨다. 溶媒를 溜去하고 殘留物을 acetone 또는 EtOH 로 再結晶한다(Table V).

抗菌力試驗

Table I, II, III, IV 및 V 에 表示한 合成物質 43種에 對한 結核菌感受性試驗을 *p*-aminosalicylic acid, INAH, *p,p'*-diethoxythiocarbanilide (DETC)를 對照로 試驗管稀釋法에^{43,44)} 따라 實施하였다.

培地—Löwenstein-Jensen medium

試驗菌株—*Mycobacterium tuberculosis* H₃₇Rv

Table VI—Antitubercular activities against *M. tuberculosis* H₃₇Rv *in vitro*.

Comp. No.	1,000	100	10	1(μ g/ml)
Control	CG	CG	CG	CG
PAS	○	○	○	○
INAH	○	○	○	○
DETC	○	○	○	+
1	+	IC	CG	CG
2	○	IC	CG	CG
3	+	+	IC	CG
4	○	IC	CG	CG
5	+	IC	CG	CG
6	○	+	IC	CG
7	○	○	+	CG
8	○	○	+	CG
9	○	○	○	IC
10	○	○	○	+
11	○	○	+	IC
12	○	+	+	CG
13	○	○	+	IC
14	○	○	+	IC
15	○	○	○	CG
16	○	○	+	IC
17	○	+	CG	CG
18	○	+	IC	CG
19	○	IC	CG	CG
20	○	+	IC	CG
21	○	○	○	IC
22	○	+	+	CG
23	○	○	○	+
24	○	○	+	+
25	○	○	○	IC
26	○	○	○	+
27	○	○	+	CG
28	○	○	+	IC
29	○	+	IC	CG
30	○	○	CG	CG
31	○	○	IC	CG
32	○	+	IC	CG
33	○	+	+	CG
34	○	○	IC	CG
35	○	+	IC	CG
36	○	+	IC	CG
37	○	○	+	IC
38	○	+	CG	CG
39	○	+	IC	CG
40	○	○	+	CG
41	○	+	IC	CG
42	○	+	+	IC
43	○	○	○	+

試料 및 對照液調製—合成物質 및 對照物質 各各 20mg을 N,N-dimethylformamide 0.1ml에 溶解하고 0.5% Sodium carboxymethylcellulose 溶液으로 稀釋하여 稀釋液 1ml中에 試料 10,000 μ g, 1,000 μ g, 100 μ g 및 10 μ g이 含有되도록 調製한다.

菌液調製—*M. tuberculosis* H₃₇Rv를 saline으로 懸탁시켜 colony數가 ml當 4~5個가 되도록 한다.

試驗管稀釋法에 의한 抗菌試驗—檢液과 對照液을 各各 濃度別로 1ml씩 試驗管에 取하고 여기에 上記 Löwenstein-Jensen medium을 9ml씩 分注하여 잘 混合한다. 85° 水浴에서 40分間 加熱하여 凝固시킨다. 直徑 4mm의 白金耳를 使用하여 菌液을 直徑 1cm의 圓形으로 接種한다. 37°에서 18~21日동안 培養하고 別途로 溶媒, 培地 및 菌液만으로 對照試驗한 結果와 比較하였다.

判定—合成物質 및 對照物質의 投與에 依한 各試驗管의 集落數를 化合物 및 濃度別로 計測하여 다음과 같이 表示하였다.

CG.....Confluent growth +.....Between 20 and 100 colonies

IC.....Innumerable discrete growth ○.....Less than 20 colonies

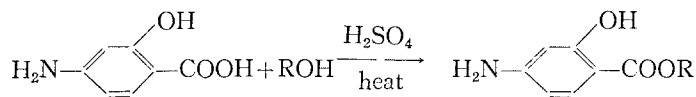
抗結核菌試驗結果는 Table VI와 같다.

考 察

1) *p*-Alkoxy aniline의 合成은 Ishidate,⁴⁾ Bucchi,⁴⁵⁾ Profft,⁴⁶⁾ Gutekunst⁴⁷⁾ 및 Doub等¹²⁾에 依하여 여러가지 方法이 提示되었으나, *p*-acetaminophen에서 *p*-acetaminophenyl alkyl ether을 製造하는 方法은 Williamson synthesis의 應用으로 큰 差異가 없다. 그러나 *p*-acetaminophenyl alkyl ether의 加水分解로 *p*-alkoxy aniline을 合成하는 方法은 Doub¹²⁾의 alkali 加水分解法이 操作의 簡便 및 高收率의 면에서 酸加水分解法보다 有利하다.

2) Alkyl *p*-aminosalicylate의 合成(Scheme 4)은 Drain⁴⁸⁾ 및 Kakemi⁴⁹⁾ 方法 共히 *p*-aminosalicylic acid와 alcohol을 少量의 濃 H₂SO₄의 存在下에 反應시키는 方法으로 通常 有機酸 ester의 製造方法과 類似하다.

그러나 90°以上에서 反應시킬 경우에는 PAS가 分解하여 脫炭酸反應이 일어나 *m*-aminophenol이 生成하므로 反應溫度에 留意하여야 한다.



Scheme 4—Synthesis of alkyl *p*-aminosalicylate.

3) Aroylisothiocyanate 가 primary 또는 secondary amine 과 反應하여 aroylthiourea 誘導體가 되고, acid hydrazide 와 反應하여 aroylthiosemicarbazide 가 生成되는 것은 addition reaction 에 依하여 이루어진다.

4) 5-Nitrofuoyl chloride 와 ammonium thiocyanate 의 反應으로 얻어지는 5-nitrofuoyliso-thiocyanate 는 粘稠性液體로서 Frank⁵⁰⁾ 및 Lipp³⁴⁾의 方法에 따라 aroylisothiocyanate 를 分離하지 않고, 直接 primary amine 또는 acid hydrazide 를 作用시켜 各各 aroylthiourea 및 aroylthiosemicarbazide 를 얻었다.

5) Table V 와 같이 合成物質은 對照物質로 使用한 PAS 및 INAH 의 結核菌阻止濃度인 1 μ g/ml 에서는 抗菌作用을 發現하지 못하였다.

結 論

1) N-Aroyl-N'-(4'-alkoxyphenyl) thiourea 誘導體 16 種, N-aryol-N'-(3'-hydroxy-4'-alkoxycarbonylphenyl) thiourea 誘導體 21 種, N-aryol-N'- α -naphthyl thiourea 誘導體 3 種 및 N-cyanoacetyl-N'-aryol thiosemicarbazide 誘導體 3 種, 計 43 種의 새로운 carbonyl thiourea 및 carbonyl thiosemicarbazide 誘導體를 合成하였다.

2) PAS ester, cyanoacetic acid hydrazide, alkoxy aniline 의 抗菌 moiety 를 考慮하여 PAS, INAH 및 DETC 를 對照物質로 試驗管稀釋法에 依하여 *M. tuberculosis* H₃₇Rv 에 對한 合成物質의 抗結核菌試驗을 遂行하였다.

3) 合成物質中 N-*p*-cyanobenzoyl-N'-4'-iso-butoxyphenyl thiourea (9), N-*p*-cyanobenzoyl-N'-*n*-amoxyphenyl thiourea (10), N-furyl-N'-4'-*n*-amoxyphenyl thiourea (15), N-*p*-cyanobenzoyl-N'-(3'-hydroxy-4'-ethoxycarbonylphenyl) thiourea (21), N-*p*-cyanobenzoyl-N'-(3'-hydroxy-4'-*n*-butoxycarbonylphenyl) thiourea (23), N-*p*-cyanobenzoyl-N'-(3'-hydroxy-4'-*n*-amoxycarbonylphenyl) thiourea (25), N-*p*-cyanobenzoyl-N'-(3'-hydroxy-4'-iso-amoxycarbonylphenyl) thiourea (26) 및 N-5-nitrofuoyl-N'-(3'-hydroxy-4'-*n*-amoxycarbonylphenyl) thiourea (43) 는 10 μ g/ml 濃度에서 結核菌의 增殖을 阻止하여 *p,p'*-diethoxy-thiocarbanilide (DETC) 와 對等한 抗結核作用을 發現함을 認知하였다.

本研究를 遂行함에 있어 始終 親切히 指導하여 주신 서울大學校 藥學大學 蔡東圭教授님께 深甚한 謝意를 表하고 實驗中 貴重한 忠告를 주신 高鉉起教授님, 鄭源根副教授님께 感謝드린다. 또한 元素分析 및 分光分析을 도와주신 朴萬基專任講師의 抗菌試驗을 도와주신 李祥義博士께 謝意를 表한다.

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