cannot be involved in the dark reactions and is probably not involved in the photo reactions either. It is especially curious that the seemingly logical reaction product (**3**) of $^1O_2$ with **1** is at best only a very minor product, if it occurs at all, in the Rose Bengal-sensitized photooxygenation of **1**[7]. There are very few instances reported where ground state oxygen ($^3O_2$) is directly implicated in what appear to be (or are) $^1O_2$ reaction products. One such example is the formation of ergosterol acetate *endo*-peroxide with $^3O_2$ and $(p\text{-}BrC_6H_4)_3N^+$[18]. The reaction of **1** → **3** on silica gel may be another.

The mechanism of formation of **3** is unclear, but a radical pathway appears to be implicated. Silica gel is known to catalyze oxidations of catechol and pyrogallol[18] and induce esr signals in adsorbed aromatic hydrocarbons[20]. It is also thought to give O_2^- in adsorbed O_2[21, 22]. The mechanism of formation of **2** is also unclear. Tetrapyrrole **2** is not obtained during the self or dyesensitized photooxygenation of **1**. Further work is in progress to elucidate the role of silica gel in these reactions and to investigate the chemistry and photochemistry of adsorbed and aggregated bilirubin.

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The Chemical Structure of Capreomycin

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Summary. The chemical structure of capreomycin, antituberculous peptide antibiotic, was revised from the results of NMR-analysis in comparison with tuberactinomycins. Capreomycin IA and IB were concluded to possess the similar amino acid sequences in their cyclic peptide moieties to those of tuberactinomycins.

The antituberculous peptide, capreomycin, was first isolated from *Streptomyces capreolus* in 1960 by Herr[1]. This antibiotic has a similar structural, as well as biochemical character, to that of viomycin, which was found in 1951[2, 3] and is in practical use as chemotherapic agent. Subsequently, capreomycin was shown to be a mixture of 4 related compounds which are named capreomycin IA, IB, IIA, and IIB[4]. The only difference in composition between I and II is attributed to the absence of β-lysine in the latter; B differs from A in exchange of 1 amino acid component, serine with alanine, having otherwise the same composition in the rest of amino acid residues.

Inspite of somewhat earlier isolation of this antibiotic, its structural elucidation for the whole molecule had not proceeded until a proposal by Bycroft et al.[5]. They deduced the structure of β-Lys→Dpr→Cpd→Ala→Dpr→Uda—[6] for capreomycin IB from some chemical evidence and an analogy to the tentative structure of viomycin, β-Lys→Dpr→Tbd→Ser→Ser→Uda—[6], which was proposed by the same authors in 1971[7].

However, shortly after this proposal, the structure of viomycin was revised as β-Lys→Dpr→Ser→Ser→Uda→Tbd—[6] by our studies on tuberactinomycin[10, 12], which is a similar antibiotic group including viomycin as one congener. This conclusion was subsequently supported by X-ray analysis by Bycroft[13]. If the similarities between capreomycin and tuberactinomycin including viomycin are accounted for in chemical, physical, and biological features, the structure of capreomycin proposed formerly might involve the wrong amino acid sequence.

In our recent studies on tuberactinomycins, the NMR-spectra of natural compounds, as well as the cyclic peptide moiety of tuberactinomycin N and O, *i.e.*, tuberactinamine N, were successfully analyzed[14]. In all the spectra, two Ser residues in the positions 3 and 4 showed significant differences in the chemical shifts and coupling patterns at α-methine, β-methylene, and α-amide protons. The results could be summarized as follows: a) the chemical shift of α-methine proton of position 3 is remarkably lower than that of position 4; b) β-methylene protons of position 3 appear as a magnetically equivalent doublet, while those of position 4 are manifested as magnetically non-equivalent two quartets; c) α-amide proton of position 4 is observed in a lower field than that of position 3. Similar phenomena were also recognized in spectra of synthetic tuberactinamine analogs, *i.e.*,

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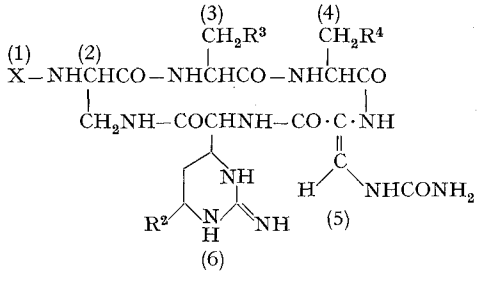
Chemical shifts from DSS for the amino acids in the positions 3 and 4 of natural and synthetic compounds

Position of amino acid residues		Tuberactinomycins				Tuberactinamine N				Capreomycins	
		A	B	N	O	Natural	Synthetic			IA	IB
							[Ala³]	[Ala⁴]	[Ala³, Ala⁴]		
3	α-CH[a]	4.86 (t)	4.85 (t)	4.84 (t)	4.85 (t)	4.84 (t)	4.68 (q)	4.79 (t)	4.55 (q)	4.79 (t)	4.58 (q)
	β-CH_2[a]	3.95 (d)	3.92 (d)	3.89 (d)	3.90 (d)	3.95 (d)	—	3.90 (d)	—	3.84 (d)	—
	α-NH[b]	8.67 (d)	8.75 (d)	8.55 (d)	8.57 (d)	8.70 (d)	8.78 (d)	8.72 (d)	8.67 (d)	8.66 (d)	8.71 (d)
4	α-CH[a]	4.32 (q)	4.31 (q)	4.31 (q)	4.30 (q)	4.32 (q)	4.33 (m)	4.28 (q)	4.22 (q)	4.30 (m)	4.33 (m)
	β-CH_2[a]	3.95 (q)	3.9 (q)	3.9 (q)	3.9 (q)	3.90 (q)	3.88 (q)	—	—	3.57∼	3.55∼
		4.17 (q)	4.15 (q)	4.16 (q)	4.18 (q)	4.20 (q)	4.20 (q)	—	—	3.89 (m)	3.89 (m)
	α-NH[b]	9.30 (d)	9.40 (d)	9.26 (d)	9.28 (d)	9.37 (d)	9.31 (d)	9.27 (d)	9.17 (d)	9.32 (d)	9.27 (d)

Abbreviations: d, doublet; t, triplet; q, quartet; m, multiplet. [a]Chemical shifts in D_2O. [b]Chemical shifts in H_2O at pH 2.5.



	X	R^1	R^2	R^3	R^4
Capreomycin IA	β-Lys or H	—	H	OH	NH_2 or β-Lys-NH
IB		—	H	H	NH_2 or β-Lys-NH
Tuberactinomycin A	CH_2CH_2CH–CH–CH_2CO	OH	OH	OH	OH
B (Viomycin)	| | | |	H	OH	OH	OH
N	NH_2 R^1 NH_2	OH	H	OH	OH
O		H	H	OH	OH
Tuberactinamine N		—	H	OH	OH
[Ala³]-		—	H	H	OH
[Ala⁴]-	H	—	H	OH	H
[Ala³, Ala⁴]-		—	H	H	H

Chemical structures of capreomycins, tuberactinomycins, and tuberactinamines.

[Ala³]-, [Ala⁴]-, and [Ala³, Ala⁴]-tuberactinamine N which were prepared in our laboratory recently[15]. The chemical shifts of the corresponding protons are listed in the Table.

Based on these findings in tuberactinomycins and tuberactinamines, we attempted to investigate the structure of capreomycin by use of NMR-analysis. Commercially available capreomycin used in our experiments was a mixture of IA and IB (ratio = 3:2)[16], without contamination with the compounds II. The components IA and IB were conveniently separated either by preparative thin-layer chromatography with a developing solvent of phenol-water-concentrated aqueous ammonia (30:10:1) or Amberlite CG-50 (Type I, 100∼200 mesh) column chromatography with gradient buffer of 0.4 *M* to 0.8 *M* ammonium acetate (pH 9.0). The amino acid composition of both IA and IB in amino acid analysis proved to be the same as reported by earlier workers[5].

The NMR-spectra of both purified materials were measured in aqueous media with a Varian Associates XL-100-15 spectrometer. For the structure of capreomycin I, we supposed from the similarities to tuberactinomycins that the sequence of Ser³-Ser⁴ in tuberactinomycins may correspond to either Ser³ (or Ala³)-Dpr⁴ or Dpr³-Ser⁴ (or Ala⁴) with the same amino acid sequences in the rest of the molecules. As expected, 2 amino acid residues in the positions 3 and 4 could be markedly distinguished (Table), *i.e.* a) the chemical shifts of α-methine protons of Ser in IA and of Ala in IB showed good agreement with those of Ser³ residue in tuberactinomycins and Ala³ residue in synthetic tuberactinamine analogs respectively; b) a doublet at δ 3.84 can be assigned to β-methylene protons of Ser residue in IA, while a multiplet indicating magnetic nonequivalency at around δ 3.73 was assigned to those of Dpr residue in each molecule of IA and IB; c) the lowest doublet in the spectra of each molecule in aqueous solution of pH 2.5 was assigned to α-amide proton of Dpr residue, while that of Ser in IA or Ala in IB appeared in practically the same region to Ser³ of tuberactinomycins or Ala³ of synthetic

tuberactinamine analogs. In addition, all the chemical shifts and coupling patterns of protons in other amino acid residues showed satisfactory similarities to those of tuberactinomycins, especially tuberactinomycin O.

From all the results shown above, it could be now concluded that the structures of capreomycins IA and IB must be revised at least in the amino acid sequences in the cyclic peptide moiety, only remaining a mode of linkage of β-lysine of the branched part undetermined whether to α-amino group of Dpr[2] or to β-amino group of Dpr[4]. In order to establish the decisive whole structure, a total synthesis of capreomycins is now in progress in our laboratory. The results on the synthetic work will be reported elsewhere.

[15] A part was presented at the 13th Symposium on Peptide Chemistry, Tokyo, 1975. Details will be reported soon elsewhere.

[16] The ratio was calculated from the integration of CH_3 protons of alanine on the basis of those of other protons on NMR-spectrum. The value was concomitant to the result of amino acid analysis of the sample.

Phosphorus-Containing Heterocycles as Fungicides: Synthesis of 2,2′-Diphenylene Chlorophosphonate and 2,2′-Diphenylene Chlorothiophosphonate

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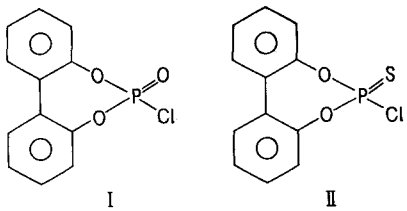
Summary. Synthesis of 2,2′-diphenylene chlorophosphonate and 2,2′-diphenylene chlorothiophosphonate is described through the interaction of 2,2′-dihydroxybiphenyl with phosphoryl chloride and thiophosphoryl chloride respectively. These compounds were screened for their fungicidal activity.

Biphenyl[1] is commonly used as fungicide for citrus fruits. Its 2-hydroxy derivative (Dowicide I)[2] is an important fungicide for the post harvest treatment of citrus fruits to reduce the incidence of rot in stored fruit. Phosphoryl and thiophosphoryl-ester linkages are frequently encountered in organic chemicals used as pesticides[3]. So it would be interesting to synthesize ring systems having phosphoryl/thiophosphoryl-ester moieties fused to biphenyl systems which are expected to increase the biological activity of organic compounds. In the present communication, we wish to report the synthesis of 2,2′-diphenylene chlorophosphonate (I) and 2,2′-diphenylene chlorothiophosphonate (II) and the comparison of their fungicidal activity with that of Dowicide I.

Reaction of phosphoryl chloride on 2,2′-dihydroxybiphenyl in refluxing benzene and in the presence of a base produced (I) in 60% yield, recrystallized from acetone, m.p. 112°. UV-spectrum exhibited λ_{max}^{MeOH} 246 nm (ε 10500). Far IR-spectrum showed strong absorption at 475 and 525 cm^{-1} attributed to P–Cl linkage. IR-spectrum showed usual absorption at 750, 810 (aromatic), 1310 (P = O) and 950, 1235 (P–O–aryl)[4]. Mass spectrum showed molecular ion peak at *m/e* 266. Other fragments were observed at 231 (M^+–Cl), 215 (M^+–Cl–O) and at 199 (M^+–2O–Cl).

Reaction of thiophosphoryl chloride on 2,2′-dihydroxybiphenyl in benzene and in the presence of pyridine gave (II) in 65% yield, recrystallized from acetone, m.p. 105°. UV-spectrum exhibited λ_{max}^{MeOH} 243 nm (ε 16800). IR-spectrum showed absorption at 750, 815 (aromatic), 960, 1240 (P-O-aryl) and 1230 cm^{-1} (P = S). Mass spectrum exhibited prominention peaks at *m/e* 282 (M^+), 266 (M^+–0), 247 (M^+–Cl), 231 (M^+–O–Cl) and 215 (M^+–2O–Cl).



I II

The fungicidal activity of the title compounds was examined against some of the important plant pathogenic fungi. The pure cultures of the organisms were obtained from the department of Plant Pathology, Punjab Agricultural University, Ludhiana and grown on Czapek's (Dox) agar medium at 25° in the presence/absence of the test chemicals[5]. The relative growth of the organisms was then compared (Table)[6].

Fungi	Inhibition (%)[a]		
	Dowicide I (standard; 1000 ppm)	Compound I (1000 ppm)	Compound II (1000 ppm)
Rhizoctonia solani	100	21	19
Fusarium oxysporum	100	17.2	22
Alternaria sp.	100	15	17
Colletotrichum sp.	100	10	7.5
Phytophthora infestans	100	5.8	4.8

[a] As compared to the controls lacking fungicides.

Biphenyl and its 2-hydroxy derivative (Dowicide I) are potent fungicides. When phosphoryl/thiophosphoryl-ester linkage is fused to biphenyl ring system in a cyclic fashion, the fungicidal activity is significantly reduced, which is contrary to the general fact that these moieties, when present in open chains, enhance their fungicidal properties[3].

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