obtained by equilibrium dialysis with tritium-labeled thalicarpine (thalicarpine-6α-7-^{3}H)[1]. This is the first report to confirm the conjecture that I, which has the necessary N—O—O triangulation for the receptor–complement feature of Zee-Cheng and Cheng (3, 4), binds to DNA.

Radioactive I (122 μCi/mg), 40 μg, was dialyzed against 40 ml of isotonic saline by placing, into 1.91 × 7.62-cm (0.75 × 3-in.) dialysis bags with 5 ml of saline, 5 ml of heat-denatured calf thymus DNA (0.7 μg/ml), 5 ml of native calf thymus DNA (0.7 μg/ml), or 5 ml of native calf thymus DNA (2.41 μg/ml). The solutions were incubated at 37° with constant shaking. At intervals of 0.25, 0.5, 1.0, 2.5, 5.0, and 18.5 hr, 100 μl was removed in duplicate and counted by liquid scintillation spectrometry. Conversions of counts per minute to disintegrations per minute were done by a computer program written by us.

It was found that the systems reached equilibrium in about 12 hr and no additional thalicarpine dialyzed from the bags into the external solution. For the same amount of DNA (3.5 μg), the binding of I was greater to denatured (3.28 nmoles/μg) than to native (0.41 nmole/μg) DNA, but the transfer rate constant for dissociation of I from DNA was less for native (3.08 hr^{-1}) than for the denatured (25.51 hr^{-1}) DNA. Therefore, native DNA was necessary for tighter binding of I.

After 24 hr, the external medium was replaced with fresh, isotonic saline and assayed as before. After an additional 24 hr, the solutions in each bag were assayed for radioactive I. The finding of persistent binding of I to native DNA suggested that this might be irreversible. Therefore, aliquots (0.2 ml) of I bound to native DNA were centrifuged in 5–20% alkaline sucrose gradients as previously described (5). Fractions from each run were analyzed for radioactivity, and the absorbance at 260 nm was determined.

In all cases, the radioactivity was found clearly separated from DNA and at the very top of the gradients. This clearly established that the binding of I to DNA was completely reversible and confirmed our previous finding that I does not lead to appreciable degradation of the DNA with which it is in contact. In similar experiments, we found that I does not bind to human serum albumin *in vitro*, but it does tightly bind to some as yet unidentified human serum component *in vivo*.

Consequently, thalicarpine is a member of a wide variety of antineoplastic compounds which bind to DNA and possess the receptor-complement feature of Zee-Cheng and Cheng (3).

Studies with DNA polymerase from *Escherichia coli* and rat liver nuclei are underway to understand further the relationship of the N—O—O triangulation feature and DNA binding by I and several of its bisbenzylisoquinoline derivatives.

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2-Ethylpyridine Derivatives with Antitubercular Properties

Keyphrases □ 2-Ethylpyridine derivatives—antitubercular activity determined □ 2-Ethylisonicotinaldehyde thiosemicarbazone—tested for antitubercular activity □ Antitubercular agents, potential—2-ethylpyridine derivatives tested

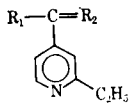
Sir:

Treatment of tuberculosis with several recently introduced chemotherapeutic agents, such as tiocarlide, ethambutol, and rifampin, remained unsuccessful in most cases refractory to standard antitubercular drugs. Therefore, it is appropriate to continue looking for potentially active agents against resistant strains of *Mycobacterium tuberculosis*. We wish to report observation of the desired activity in a 2-ethylpyridine derivative, a member of a group previously reported as possessing tuberculostatic properties (1–3). The observation was made while screening the following types of compounds: 2-ethylisonicotinic acid thiosemicarbazide, some *N*-arylsulfonyl substituted 2-ethylpyridineisonicotinic acid hydrazides, and the thiosemicarbazone of the corresponding 2-ethylisonicotinaldehyde. The structures of these compounds and pertinent references are summarized in Table I.

Tuberculostatic activity was examined in liquid (Proskauer–Beck) and on solid (Lowenstein–Jensen) media. In one set of experiments, the compounds were tested in a series of concentrations (0.1, 1.0, 10, and 100 μg/ml medium) against a sensitive strain of *M. tuberculosis* H37 Rv. In another set, a strain resistant to standard antitubercular agents was used as the test organism and exposed to the same concentrations.

As in previous work, *in vivo* activity was routinely checked along with the bacteriostatic tests. *In vivo* activity was tested in mice infected by intravenous inoculation with *M. bovis* Ravenel Rv. Controls survived this treatment for 19–22 days. A compound

Table I—2-Ethylpyridine Derivatives Tested for Antitubercular Activity



Compound	R_1	R_2	Reference
	(*a*): —NHNH—X	=O	
1	X: —$CSNH_2$		5
2	X: —O_2S—C_6H_4-*p*-Y		4
	Y: —H		
3	Y: —CH_3		5
4	Y: —Cl		5
5	Y: —NO_2		5
6	Y: —NH_2		6
7	Y: —$NHCOCH_3$		5
8	(*b*): —H	=$NNHCSNH_2$	4, 6, 7

was considered to be active *in vivo* if a 10-mg/kg ip dose, repeated daily through 14 days, increased the mean survival period by a statistically significant increment. Statistical significance was assessed by comparing reaction time ratios according to Litchfield (8).

The results from *in vitro* experiments are shown in Table II and those from *in vivo* tests are given in Table III. The data in Table II show that the thiosemicarbazide and most *N*-arylsulfonyl hydrazides of 2-ethylisonicotinic acid (Compounds 1 and 4–7) possess rather weak tuberculostatic activities against sensitive *M. tuberculosis*, since these compounds inhibited bacterial growth only at the highest concentration level. Two hydrazides, the unsubstituted *N*-phenylsulfonyl derivative (Compound 2) and the *N*-(*p*-tolyl)sulfonyl derivative (Compound 3), were inactive in these experiments. With the resistant strain, all but one of the compounds tested were inactive. The outstanding exception was Compound 8, 2-ethylisonicotinaldehyde thiosemicarbazone. This compound is a strong tuberculostatic, since it inhibited the growth of the resistant strain at a concentration of 1.0 μg/ml and that of the sensitive microorganism at 0.1 μg/ml.

Table III—Antitubercular Action of 2-Ethylisonicotinic Acid and 2-Ethylisonicotinaldehyde Derivatives: *In Vivo* Effects

Compounds Tested[a]	ET_{50}[b], Days	*RR*[b]	*fRR*[b]
1	33.5	1.52	1.46
2	25	1.13	1.15
3	37	1.68	1.23
4	33.5	1.52	1.46
5	25	1.13	1.15
6	36	1.63	1.105
7	51	2.31	1.09
8	36.5	1.66	1.365
Streptomycin	36	1.63	1.105
Aminosalicylic acid	37	1.68	1.23
Isoniazid	51	2.31	1.09
Controls	20.5	—	—

[a] Numbers correspond to compounds listed in Table I; duration of treatment was 14 days with daily doses of 10 mg/kg. Number of animals treated was 10 per group with 10 animals as controls. [b] Statistics according to Litchfield (8); ET_{50} = median effective time, *RR* = reaction time ratio, *fRR* = factor or reaction time ratio; criterion for statistical significance of test was $RR > fRR$.

In vivo, all compounds, with two exceptions, significantly lengthened the mean survival period of mice infected with *M. bovis* (Table III). Compound 2, *N*-phenylsulfonyl hydrazide, was ineffective both *in vitro* and *in vivo;* Compound 5, *N*-(*p*-nitrophenyl)sulfonyl hydrazide, was inactive *in vivo* but was slightly active *in vitro*. Another hydrazide, the *N*-(*p*-tolyl)sulfonyl hydrazide (Compound 3) behaved in an opposite manner, with activity being observed only *in vivo*.

2-Ethylisonicotinaldehyde thiosemicarbazone was moderately effective against *M. bovis in vivo*. Its relative potency was comparable to those of streptomycin and aminosalicylic acid.

The strongest *in vivo* effect was obtained with N^1-(*p*-acetamidophenyl-N^2-(2-ethylisonicotinoyl) hydrazide (Compound 7) (about 1.4 times as potent as streptomycin), yet this compound was only weakly tuberculostatic and altogether inactive against resistant *M. tuberculosis*.

Table II—Antitubercular Action of 2-Ethylisonicotinic Acid and 2-Ethylisonicotinaldehyde Derivatives: *In Vitro* Effects

Medium	Organism[a]	Concentration of Agent, μg/ml	Effect[b] of Compounds Listed in Table I								Effect[b] of Antitubercular Drug[c]		
			1	2	3	4	5	6	7	8	S	A	I
Liquid[d]	Sensitive	0.1	−	−	−	−	−	−	−	+	+	+	+
		1.0	−	−	−	−	−	−	−	+	+	+	+
		10	−	−	−	−	−	−	−	+	+	+	+
		100	+	−	−	+	+	+	+	+	+	+	+
	Resistant[e]	0.1	−	−	−	−	−	−	−	−	−	−	−
		1.0	−	−	−	−	−	−	−	+	−	−	−
		10	−	−	−	−	−	−	−	+	−	−	−
		100	−	−	−	−	−	−	−	+	−	−	−
Solid[f]	Sensitive	0.1	−	−	−	−	−	−	−	+	+	+	+
		1.0	−	−	−	−	−	−	−	+	+	+	+
		10	−	−	−	−	−	−	−	+	+	+	+
		100	+	−	−	+	+	+	+	+	+	+	+
	Resistant[e]	0.1	−	−	−	−	−	−	−	−	−	−	−
		1.0	−	−	−	−	−	−	−	+	−	−	−
		10	−	−	−	−	−	−	−	+	−	−	−
		100	−	−	−	−	−	−	−	+	−	−	−

[a] *M. tuberculosis* H37 Rv. [b] + = growth inhibition; − = no inhibition. [c] S = streptomycin, A = aminosalicylic acid, and I = isoniazid. [d] Proskauer–Beck medium. [e] Resistant to drugs listed in Footnote *c*. [f] Lowenstein–Jensen medium.

In summary, the experiments reported in this communication revealed a strong tuberculostatic activity for 2-ethylisonicotinaldehyde thiosemicarbazone. The growth-inhibiting action of this compound extends against a strain of *M. tuberculosis* resistant to streptomycin, aminosalicylic acid, and isoniazid. In addition, 2-ethylisonicotinaldehyde thiosemicarbazone is also moderately effective in an *in vivo* test and may be considered as a promising candidate as an antitubercular drug.

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BOOKS

REVIEWS

The Alkaloids, Volume 3, A Specialist Periodical Report. By J. E. SAXTON, Senior Reporter. The Chemical Society, Burlington House, London, WIV OBN, England, 1973. ix + 337 pp. 14.5 × 22 cm. Price £8.50.

The third volume on alkaloids in this series of Specialist Reports sets out to review the literature published in the field of alkaloid chemistry for the period July 1971 to June 1972. This issue includes for the first time a summary of recent developments in the chemistry of the steroidal alkaloids of the *Solanum* and *Veratrum* groups. In this chapter, the salient literature references from the beginning of 1970 have also been included although the emphasis has been placed on the period of review.

This volume again opens with a survey of current interest in biosynthesis followed by 16 chapters describing developments in structural and synthetic chemistry. In most cases, pertinent spectroscopic and pharmacological data, as they pertain to the alkaloids, are discussed.

There is no doubt that all who have an interest in the alkaloids will be indebted to the authors of these reviews for undertaking this task. With few exceptions, the authors have made it possible to keep abreast of the major new developments in this vast field of natural products chemistry. Structural formulas are plentiful and accurately presented which make for easy reading. An author index is also included.

I think that this Specialist Report will appeal particularly to the pharmaceutical scientist and researcher interested in pharmacologically active and clinically useful alkaloids. It should also find a place on every library shelf as an aide to teachers and researchers.

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Organophosphorus Chemistry Volume 4. S. TRIPPETT, Senior Reporter. The Chemical Society, Burlington House, London, WIV OBN, England, 1973. xi + 305 pp. 14 × 22 cm. Price $7.50.

The literature published between July 1971 and June 1972 is reviewed and summarized in this volume. The study of stable quinquecovalent phosphoranes and their pseudorotation phenomena and the application of molecular orbital calculations to studies of bonding in phosphorus compounds are two areas of developing interest that are covered.

Staff Review

CTFA Cosmetic Ingredient Dictionary 1973. Edited by NORMAN F. ESTRIN. The Cosmetic, Toiletry, and Fragrance Association, Inc., 1625 Eye Street, N.W., Washington, DC 20006, 1973. xiv + 253 pp. 21.5 × 27.5 cm.

Information on materials utilized in the manufacture of cosmetics is compiled in this publication. To make cosmetic ingredient nomenclature more uniform, the Cosmetic, Toiletry, and Fragrance Association has designated or adopted preferred names for commonly used ingredients. For the most part, these names do not conflict with official nomenclature, *e.g.*, USAN or NF, although some deviations are found.

The bulk of the book consists of short monographs for cosmetic ingredients which include the adopted name, CAS registry number, structural formulas, reference sources, and a listing of other names. A listing of chemical/trade names referenced to the adopted name completes the book.