Table I—Disintegration Time (Minutes) of Prepared Tablets

Substance	Starch Disintegrant	Mean Disintegration Time, 10% Starch	Mean Disintegration Time, 15% Starch
Aspirin	Potato	5.35	6.12
	Trapa	6.49	4.30
	Wheat	7.0	5.20
	Maize	6.0	4.10
Calcium carbonate	Potato	8.1	6.0
	Trapa	8.30	6.0
	Wheat	9.0	8.0
	Maize	7.0	5.0
Sulfathiazole	Potato	4.0	4.3
	Trapa	4.4	3.3
	Wheat	4.56	5.3
	Maize	6.1	4.0
Sodium bicarbonate	Potato	12.2	10.2
	Trapa	12.45	9.2
	Wheat	10.1	13.2
	Maize	9.3	8.3

graph in the India Pharmacopoeia (2). Trapa starch is tasteless, odorless, and white in color with a fine texture. The starch consists of polyhedral or rounded granules about 10–40 μm. The gelatinization temperature of the starch was found to be at 80°, as observed by the method suggested by Radley (3). Chemical studies gave the following results: total reducing sugars, 67.5%; protein, 7.58%; and crude fiber, 2.34%. Identification of sugars was carried out by a paper chromatographic method, and glucose and maltose were identified. Among the metals, iron was detected.

For the present investigation, four starches were selected as tablet disintegrants: trapa, potato, maize, and wheat. By using each of these starches separately, tablets of aspirin, sulfathiazole, calcium carbonate, and sodium bicarbonate were prepared[2]. Granules of calcium carbonate, sulfathiazole, and sodium bicarbonate were prepared by moist granulation, while aspirin granules were prepared by dry granulation. All starches were used in 10 and 15% concentrations. The tablet weight was kept constant at 0.400 g, and the tablet hardness was kept as near to 5.5 kg/cm^2 as possible by a hardness tester[3].

Appearance—All tablets prepared with trapa starch had an excellent appearance and were glossy.

Uniformity of Weight—This parameter conformed to India Pharmacopoeia (2) specifications.

Compression Ratio—The ratio was found to be nearly uniform in all the tablets (4). It ranged from 0.87 to 0.88 for aspirin, from 0.87 to 0.89 for sulfathiazole, from 1.12 to 1.13 for sodium bicarbonate, and from 1.34 to 1.37 for calcium carbonate.

Disintegration Time—The USP XV (5) apparatus and method were used to determine disintegration time. The tablets prepared with trapa starch at 15% concentration showed a remarkable decrease in disintegration time in contrast to other starches at 15% concentration as well as its own 10% concentration. The mean disintegration time of different tablets is given in Table I.

By using an analysis of variance technique, it was observed that at the 1% level of significance the 15% concentration gave lower disintegration times than the 10% concentration for all four starches. By using Scheefe's (6, 7) 95% probability interval estimate, it was observed that tablets with trapa starch had lower disintegration times than those containing potato or wheat starch but greater disintegration times than those containing maize starch.

Thus, trapa starch proved efficient as a disintegrating agent in 15% concentration. The potentialities of starch from *T. bispinosa* as a substitute for currently used starches are considerable.

[2] Tablets were prepared on a Unimake single-stroke, four-punch tablet machine using 0.94-cm (0.37-in.) punches of standard concavity.

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Binding of a New Antitumor Agent, Thalicarpine, to DNA

Keyphrases □ Thalicarpine (antineoplastic alkaloid)—binding to calf thymus DNA, N—O—O triangulation structure □ Antineoplastic alkaloids with N—O—O triangulation structure—binding of thalicarpine to calf thymus DNA □ Binding of antineoplastic alkaloids to calf thymus DNA—radiolabeled thalicarpine □ Equilibrium dialysis—determination, binding of radiolabeled thalicarpine to calf thymus DNA

Sir:

Thalicarpine (I), a new antineoplastic alkaloid with novel structure, is in initial clinical trial in our service (1).

We previously described the multiplicity of action of thalicarpine against L-1210 mouse leukemia cells in culture (2). We reported that we could not detect any binding of thalicarpine to calf thymus DNA *in vitro* by UV spectroscopy.

We now wish to present evidence for DNA binding

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