aminoester could not be prepared in the solid state. Infrared spectral bands in cm.$^{-1}$: 3250(w), 1720(s), 1635(s), 1595(s), 1445(s), 1360(m), 1233(s), 1165(s), 1083(m), 1060(m), and 1036(m).

Ethyl cis-Hexahydroanthranilate (III).—A mixture of 10 Gm. of ethyl anthranilate in 150 ml. of absolute ethanol and 8 Gm. of 5% rhodium on alumina was stirred in a Parr pressure reaction apparatus at 500 p.s.i. and at temperatures up to 85° for a total of 10 hours over a 2-day period. Removal of catalyst and solvent gave an oil which distilled at 115–119° (20 mm.). Huenig and Kahanek report 103–104° (11 mm.) (4). The yield of hexahydro ester was 7.3 Gm. (70.1%). For analysis, the previously unreported HCl salt of III was prepared in the usual manner with dry ether and HCl gas and melted at 131–133° after two crystallizations from acetone/alcohol.

Anal.—Calcd. for $C_9H_{18}ClNO_2$: C, 52.05; H, 8.67; N, 6.74. Found: C, 51.82; H, 8.71; N, 6.62.

The free acid was prepared by refluxing III in plain water, followed by evaporation. The *cis*-hexahydroanthranilic acid so obtained was recrystallized from alcohol/water, m.p. 233–234°. Huenig and Kahanek (4) report 235° for the *cis* acid; the *trans* acid, also prepared by plain water hydrolysis (1), melts at 274°.

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Antistaphylococcal Activity of Seeds of *Psoralea corylifolia*

By K. N. GAIND, R. N. DAR, and R. N. KAUL

The petroleum ether extract of the seeds of *Psoralea corylifolia* Linn. inhibits the growth of *Staphylococcus aureus*, in the concentration of 10 mcg./ml. From the extract a pure compound, $C_{12}H_{16}O$, b.p. 155°/0.3 mm., has been isolated by chromatography and subsequent fractional distillation. It inhibits the growth of *S. aureus* in the concentration of 0.5 mcg./ml.

PSORALEA CORVLIFOLIA Linn. (*Leguminosae*) is a common herbaceous weed, grows throughout the plains of India, and is commonly known as babchi. The seeds of this plant have long been used in the indigenous system of medicine for leprosy, inflammatory diseases of the skin, leucoderma, psoriasis, as an anthelmintic, a diuretic and a diphoretic in febrile conditions (1). In southern India, the drug is widely used as a stomachic, deobstruent, and in various cutaneous diseases (2). The seeds have an estrogenic effect on certain laboratory animals (3). Jois and Manjunath (4) and Seshadri and Venkattarao (5) isolated o fixed and a volatile oil, psoralen and iso-psoralen. Chakravarti *et al.* (6) isolated a new crystalline compound, psoralidin, from the pericarp. Gupta *et al.* (7) recently reported the antistaphylococcal activity of the petroleum ether extract of the seeds in the concentration of 2–4 mcg./ml. Jois *et al.* (8) obtained from a petroleum ether extract of the seeds a fixed oil, psoralen, and iso-psoralen. In this investigation, these components did not show activity against *Staphylococcus aureus*. Volatile oil from the seeds has not revealed appreciable activity (7). A systematic investigation of the seeds was undertaken and an attempt made to isolate and characterize the antistaphylococcal principle.

MATERIAL AND METHODS

Test Organism.—*S. aureus* (Oxford culture) was employed for testing the activity of the various fractions. The organism was maintained on nutrient agar slants from which regular subculturing was done in nutrient broth. A 24-hour suspension of this organism in broth was used for all subsequent investigations.

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Minimum Inhibitory Concentration.—The antistaphylococcal activity of the various fractions (Table I) was tested by serial dilution method. A solution (10 mg./ml.) of the test compound was prepared in alcohol (90%). One-half milliliter of the dilution was then transferred to 4.5 ml. of sterile nutrient broth (pH 7.2–7.4) from which 0.5 ml. of the dilution was further transferred to 4.5 ml. of the broth, etc., until a varied range of concentrations was obtained in the broth. The total volume was kept at 5 ml. Various dilutions of the test compound ranging from 0.1 to 100 mcg./ml. were obtained. The solution of the test compound

TABLE I.—ANTISTAPHYLOCOCCAL ACTIVITY OF VARIOUS CHROMATOGRAPHIC FRACTIONS OF PETROLEUM ETHER EXTRACT OF *P. corylifolia* SEEDS

Eluents	Physical Appearance	Antistaphylococcal Activity[a]
Petroleum ether (1–18)	Dark brown liquid	−
10% Benzene in petroleum ether (19–29)	Light green liquid	−
50% Benzene in petroleum ether (30–40)	Viscous green liquid	+
Benzene (41–53)	Viscous green liquid	+
10% Ether in benzene (54–57)	Viscous brown liquid	−
50% Ether in benzene (58–67)	Viscous brown liquid	−
Ether (68–75)	Viscous brown liquid	−
10% Alcohol in ether (76–79)	Yellow solid mass	−
50% Alcohol in ether (80–83)	Yellow solid mass	−
Alcohol (84–90)	Yellow solid mass	−

[a] −, No inhibition; +, inhibition.

in alcohol on dilution with broth gave slight turbidity. However, concentrations below 100 mcg./ml. were all clear solutions. The tubes were then inoculated with 0.05 ml. of the suspension of *S. aureus* in broth and incubated at 37°. These were then examined after 24 hours for visible growth.

EXPERIMENTAL

The seeds of the drug were coarsely powdered and subjected to successive extractions with solvents in a Soxhlet apparatus. The per cent residues were: petroleum ether, 8.6; chloroform, 5.2; and alcohol, 2.8.

The petroleum ether extract inhibited the growth of *S. aureus* in the concentration of 10 mcg./ml. Since the chloroform and alcoholic extracts did not show antistaphylococcal activity, these extracts were not taken up for further study.

Isolation of Antistaphylococcal Principle.—Ten grams of petroleum ether extract was chromatographed over 100 Gm. of alumina (Brockmann grade I) using, successively, petroleum ether, benzene, ether, alcohol, and their appropriate mixtures (Table I) as elution solvents. All the solvents were previously dried and purified. Ninety 30-ml. fractions were collected. The solvents were removed *in vacuo*, and the antistaphylococcal activity of the various fractions recorded (Table I). After the elution, the chromatographic column showed the presence of six different bands, which were extruded separately and refluxed with methyl alcohol on a water bath. The residues were tested and did not show antistaphylococcal activity.

The eluates consisting of petroleum ether–benzene mixture (1:1) were pooled and designated as fraction A. After refrigerating for a few days, it deposited needle-shaped crystals which were washed with cold petroleum ether to remove the adhering liquid. A white crystalline compound was obtained which on repeated crystallization from hot dilute alcohol gave a pure compound. It was identified as psoralen and did not show activity against *S. aureus*. The mother liquor was further concentrated, and 1 Gm. of this liquid was again passed through a column containing 5 Gm. of alumina. The column was then eluted with petroleum ether–benzene mixture (1:1). The solvent was removed *in vacuo*, and the residual liquid was distilled under reduced pressure; the fraction B, b.p. 155°/0.3 mm., was collected. It was of a pale, viscous oily nature and inhibited the growth of *S. aureus* in the concentration of 0.5 mcg./ml.

Anal.—Calcd. for $C_{12}H_{16}O$. Found: C, 82.22; H, 8.85, mol. wt. (Rast) 186.

The benzene eluates were also mixed together and did not deposit crystals when cooled. The liquid was distilled under reduced pressure and the fraction, b.p. 155°/0.3 mm., was obtained. The compound on analysis was identical to B ($C_{12}H_{16}O$) and had equal inhibition against *S. aureus*.

The activity of compound B was also tested against *S. aureus* resistant to penicillin, streptomycin, chloramphenicol, and tetracycline, in which it was equally effective.

A survey of the literature has revealed that compound B ($C_{12}H_{16}O$) has not been reported previously in *P. corylifolia*. The preliminary characterization tests have revealed the aldehyde nature of this compound; therefore, the name psoralaldehyde is proposed for it. Detailed work about its characterization and constitution will be communicated later.

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Semiautomatic System for Timing Rotarod Performance

By NATHAN WATZMAN, HERBERT BARRY, III, JOSEPH P. BUCKLEY, and WILLIAM J. KINNARD, JR.

An electronic circuit automatically stops the timer when the animal falls to a platform beneath the rod. This system improves the accuracy and convenience of recording rotarod performance time. The investigator may test several animals simultaneously with only periodic observation.

THE ROTAROD is a device which has been used extensively for the evaluation of drug effects on the central nervous system. Rats and mice perform a task similar to log-rolling; neurological deficit induced by a drug is indicated by the inability of an animal to remain on the rod for a specified period of time (1). Various types of central nervous system depressants (2, 3) and stimulants (4) may be evaluated and compared by the increase or decrease in performance times they produce.

The rod is generally divided into compartments by thin circular disks, larger in diameter than the roller, so that several animals can be tested simultaneously. The experimenter starts a timer as soon as he places each animal on the rod and stops the timer as soon as he sees the animal fall to the platform beneath.

In situations where as many as six animals are being tested at one time, it is necessary for the experimenter to give his undivided attention. Under these circumstances, it is quite possible that, if more than one animal falls in quick succession, the experimenter might not be able to observe each fall

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