Examination of the Oil.—The red basil oil which was produced by the same method as the white basil oil, had a strong spicy odor and its color was reddish. It had the following physicochemical constants: specific gravity (20°), 0.9111; optical rotation, −10.75°; refractive index (20°), 1.4830; acid number, 6.5; and ester number, 14.5.

Chromatographic Analysis

The oil was investigated the same way as the white basil oil. It had the same chemical composition (Fig. 1) except for the following:

Methyl Chavicol.—This could be detected by thin-layer chromatography against authentic methyl chavicol.

Cinnamic Acid Ester.—Cinnamic acid could be detected by its hydroxamic acid as described in the white basil oil investigation. The isolation of the ester on the column or on the chromatoplates was unsuccessful.

Safrol.—Could not be detected.

Eugenol.—Could be easily isolated on the column.

CONCLUSION AND DISCUSSION

The investigation of the two Egyptian basil oils undertaken in this study, revealed the occurrence of terpineol, linalool, cineole, eugenol, esterified geraniol, citronellol, linalool, and terpineol with acetic and formic acids (geranyl and/or citronellyl acetate, linalyl and/or terpinyl acetate, citronellyl formate), and a sesquiterpene alcohol (nerolidol?) in both types of oils. In addition, the white type contained methyl cinnamate and (safrol?). The red type contained methyl chavicol and traces of cinnamic acid ester. The presence of terpineol, citral, and safrol has not been reported before in the *O. basilicum* oils. Therefore the basil oil types outlined by Guenther (2) and Gildemeister and Hoffmann (3) cannot be applied in arranging these two oil types in any of the reported types. This viewpoint is supported by the occurrence of citral in other *Ocimum* species, *e.g.*, *O. canum* Sims (2, 3), *O. gratissimum* L., and *O. menthaefolium* Hochst. (3). The occurrence of constituents in the Egyptian basil oils, which have not been reported previously, and their differences from basil oil samples of European origin analyzed at the same time, lead to the possibility of arranging these two Egyptian basils in separate chemical or physiological races [Dillemann (15), Rowson (16), Huerlimann (17)], the category to include those plants indigenous to and/or cultivated in Egypt. Further study of the oils of these plants in different periods of plant growth is recommended to ascertain the occurrence and percentage of their chemical constituents which may change according to climatic, edaphic, and genetic factors. These qualitative and quantitative studies are being carried out.

REFERENCES

(1) Hegi, G., "Illustrierte Flora von Mittelleuropa," Vol. 5/4, 2nd ed., J. F. Lehmann, München, Germany, 1939, p. 2267.
(2) Guenther, E., "The Essential Oils," Vol. 3, 3rd ed., D. Van Nostrand Co., Inc., Princeton, N. J., 1958, pp. 399–433.
(3) Gildemeister, E., and Hoffmann, F., "Die Aetherischen Oele," Vol. 7, 5th ed., Akademische Verlag, Berlin, Germany, 1961, pp. 478–516.
(4) Tackholm, V., "The Student's Flora of Egypt," The Anglo-Egyptian Bookshop, Cairo, Egypt, 1956, p. 140.
(5) Ascherson, F., and Schweinfurth, G., "Illustration de la flore d'Egypte," Le Claire, 1887, p. 120.
(6) Hoerhammer, L., and Wagner, H., *Deut. Apotheker-Ztg.*, **101/26**, 779(1961).
(7) Hoerhammer, L., Richter, G., and Wagner, H., *J. Chromatog.*, **10**, 108(1963).
(8) Hoerhammer, L., private communication, Institut für Pharmazeutische Arzneimittellehre der Universität München.
(9) Guenther, E., "The Essential Oils," Vol. 2, 2nd ed., D. Van Nostrand Co., Inc., Princeton, N. J., 1958, p. 775.
(10) *Ibid.*, p. 275.
(11) Vogel, A., "A Textbook of Practical Organic Chemistry," 3rd ed., Longmans, Green, London, 1959, p. 344.
(12) Hais, M., and Macek, K., "Handbuch der Papierchromatographie," Vol. 1, VEB Gustav Fischer Verlag, Jena, Germany, 1958, p. 770.
(13) *Ibid.*, p. 214.
(14) Miller, J. M., and Kirchner, J. G., *Anal. Chem.*, **25**, 1107(1953).
(15) Dillemann, G., *Planta Med.*, **8**, 263(1960).
(16) Rowson, J. M., *ibid.*, p. 243.
(17) Huerlimann, H., *ibid.*, p. 216.

Synthesis and Antitubercular Activity of Isonicotinoyl and Cyanoacetyl Hydrazones

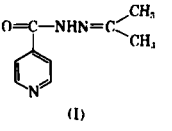
By DIPTISH CHAKRAVARTY, ARUN BOSE, and SAMIR BOSE

Seven isonicotinoyl hydrazones and four cyanoacetyl hydrazones were synthesized. Their antitubercular activities *in vitro* and *in vivo* were evaluated.

THE ANTIMICROBIAL activity of isoniazid against tubercle bacilli depends on the amount of free and unaltered isoniazid (1). The observations of Fox and Gibas (2) show that one or both of the hydrogen atoms attached to nitrogen in the hydrazine moiety of isoniazid may be replaced by a variety of groups with little loss of activity.

Received June 7, 1963, from the Research Division, Smith, Stanistreet and Co., Calcutta, India.
Accepted for publication September 7, 1963.

Fox and Gibas (3) have shown that isonicotiny hydrazine when reacted with acetone gives 1-isonicotinyl-2-iso-propylidene hydrazine (I)



(I)

which proved to be very active against tubercle bacilli. These authors (3) have investigated systematically alkylidene derivatives of isonicotinyl hydrazone with the twofold view of dis-

TABLE I.—ISONICOTINOYL HYDRAZONES

Compd.	R	Crystallizing Solvent	Appearance	M.p., ° C.	Yield, %	Mol. Formula	N, % Calcd.	N, % Found
AT–1	(*o*)-OHC_6H_4CH	Ethanol	Colorless needles	243–244	83	$C_{13}H_{11}N_3O_2$	17.43	17.41
AT–2	(*m*)-OHC_6H_4CH	Methanol	Cream-colored rods	255–257	86	$C_{13}H_{11}N_3O_2$	17.43	17.40
AT–3	(*p*)-OHC_6H_4CH	Ethanol	Light cream-colored prisms	275–277	85	$C_{13}H_{11}N_3O_2$	17.43	17.41
AT–4	(*p*)-$(CH_3)_2NC_6H_4CH$	Dil. ethanol	Golden yellow plates	201–202	93	$C_{15}H_{16}N_4O$	20.88	20.85
AT–5	$C_6H_5—C(CH_3)$	Dil. ethanol	Colorless rods	172–173	60	$C_{11}H_{14}N_2O$	16.67	16.65
AT–6	(*p* and *m*) $(OCH_3)_2C_6H_3CH$	Dil. ethanol	Colorless prisms	188–189	70	$C_{18}H_{18}N_2O_3$	14.74	14.70
AT–7	$C_6H_5CH{:}CH{\cdot}CH$	Ethanol	Cream-colored needles	200–202	80	$C_{15}H_{12}N_2O$	16.73	16.74

TABLE II.—CYANOACETYL HYDRAZONES: $CNCH_2CONHN = R$

Compd.	R	Crystallizing Solvent	Appearance	M.p., ° C.	Yield, %	Mol. Formula	N, % Calcd.	N, % Found
AT–8	C_6H_5CH	Ethanol	Colorless rods	178–180	64	$C_{10}H_9N_3O$	22.46	22.45
AT–9	(*o*)-OHC_6H_4CH	Ethanol	Light yellow needles	166–168	69	$C_{10}H_9N_3O_2$	19.54	19.50
AT–10	(*p*)-OHC_6H_4CH	Dil. ethanol	Cream-colored plates	210–212	74	$C_{10}H_9N_3O_2$	19.54	19.55
AT–11	(*p*)-OH, (*m*)-$OCH_3C_6H_3CH$	Isopropanol	Light yellow plates	202–204	69	$C_{11}H_{11}N_3O_3$	18.03	18.04

covering superior tuberculostat. Since the isopropylidene group is a branched chain substitution, the study was extended to the straight chain alkylidenes; the 1-isonicotinyl-2-alkylidene hydrazine type, whether straight or branched chains, were actively tuberculostatic *in vivo*.

On the fragile basis of the similarity of interatomic distances between the nitrogen atoms and hydrazine moiety of cyanoacetic acid hydrazide and isoniazid, Valdecasas (4) and his co-workers conceived the idea of the probable tuberculostatic activity of cyanoacetic acid hydrazide and reported that it was indeed a remarkable tuberculostat in experimental and clinical tuberculosis comparable to isoniazid.

Shchukina, *et al.* (5), reported the synthesis of a number of isonicotinyl hydrazones from aldehydes and ketones as antitubercular agents. Their biological tests indicated that some of these isonicotinyl hydrazones are more active *in vivo* and far less toxic in animals (mice). These authors lay great emphasis on the high toxicity of isonicotinic acid hydrazide believing that the new drug does not possess a large margin of safety.

The recent results of biological tests indicate that if too much emphasis is placed on the value of isonicotinic acid hydrazide alone as a chemotherapeutic agent for the treatment of tuberculosis overlooking the possibilities of its derivatives, there is a great danger that some most interesting and valuable compounds might be missed.

This paper reports the synthesis and chemotherapeutic evaluation of antitubercular activity of (*a*) different isonicotinyl hydrazones and (*b*) cyanoacetyl hydrazones.

EXPERIMENTAL

General Procedure for the Preparation of Isonicotinoyl Hydrazones.—Isonicotinic acid hydrazide (0.05 mole) was dissolved in a minimum quantity of distilled water. To this solution, the aldehyde or ketone (0.05 mole), either as such (compound AT–1) or dissolved in a minimum quantity of ethanol (compounds AT–2, AT–3, AT–4, AT–5, AT–6, and AT–7) was added with stirring. In some cases the crystals separated within 5 minutes or after about 24 hours. However, in all cases, the crystals were separated after 24 hours by filtration. The solids were crystallized from suitable solvents and dried *in vacuo* over calcium chloride. The characteristics of the compounds synthesized following the above procedure are recorded in Table I.

General Procedure for the Preparation of Cyanoacetyl Hydrazones.—Cyanoacetic acid hydrazide (0.05 mole) was dissolved in water (25 ml.). To this solution under stirring was added the aldehyde (0.05 mole) either as such (compounds AT–8 and AT–9) or dissolved in a minimum quantity of

TABLE III.—RESULTS OF *In Vitro* TEST OF ALL TEST COMPOUNDS ON H_{37} Rv STRAIN[a]

Compd.	Response[b]
AT-1	++
AT-2	±
AT-3	++
AT-4	++
AT-5	+
AT-6	+
AT-7	±
AT-8	±
AT-9	±
AT-10	●
AT-11	±
INH	++
Control[c]	−

[a] Dose (mcg./ml.) was 0.1 and 0.2. [b] ++ = Complete inhibition of growth; + = partial inhibition of growth; ± = no inhibition of growth; − = full growth. [c] No drug.

ethanol (compounds AT-10 and AT-11). The crystals, which were filtered, separated within 24 hours at room temperature. They were crystallized from suitable solvents and dried *in vacuo* over calcium chloride. The characteristics of the compounds synthesized following the above method are shown in Table II.

Cyanoacetic Acid Hydrazide.—Hydrazine hydrate (25 ml., 60% w/v) was added dropwise to the stirred and cooled (below 5°) ethyl cyanoacetate (22 Gm.) and stirred for 3 hours. The precipitated colorless microcrystalline solid was filtered and dried *in vacuo* over calcium chloride. Yield 15 Gm.; m.p. 110–112°. The product develops color if the reaction is carried out at a higher temperature than 5°.

Anal.—Calcd. for $C_3H_5N_3O$: N, 42.42. Found: N, 42.40.

PHARMACOLOGICAL EVALUATION

Both *in vitro* and *in vivo* tests were done according to the method worked out by Sah and Peoples (6). *Mycobacterium tuberculosis* strain $H_{37}Rv$ maintained on Lowensten Jensen media and albino rats of our own breed were used.

In Vitro Test.—Compounds were incorporated with Lowensten Jensen medium in series and were kept in an inspissator at 80° for 1 hour for 3 consecutive days. Thereafter, they were inoculated with 0.1 ml. of 10-day-old culture of *M. tuberculosis* grown in Dubos medium, incubated at 37° for 3 weeks. Control tubes containing only strain culture were maintained for comparison.

Table III indicates the dose response of different

TABLE IV.—ACUTE TOXICITY OF TEST COMPOUNDS ON ALBINO RATS[a]

Compd.	Mortality
AT-1	6/6
AT-2	2/6
AT-3	0/6
AT-4	0/6
AT-5	0/6
AT-6	6/6
AT-7	2/6
AT-8	2/6
AT-9	1/6
AT-10	0/6
AT-11	2/6

[a] Six rats with an average body weight of 100 Gm. each; maximum oral dose, 1 Gm./Kg.

TABLE V.—*In Vivo* TEST ON ALBINO RATS WITH VARIED DOSE[a] AND CHANGES IN INTERNAL ORGANS

Compd.	Animals, No.[c]	Days on Test (with each dose)	Mortality, %: (a)[a]	(b)[a]	Liver[b]: (a) 0.2%	(b) 0.5%	Spleen[b]: (a) 0.2%	(b) 0.5%	Lungs[b]: (a) 0.2%	(b) 0.5%
AT-1	4 and 3	40	25	33	+	+	++++	+	+	+
AT-2	2 and 2	28	0	0	+	+	+	+	+	+
AT-3	4 and 3	40	25	33	+	+	+	+	+	+
AT-4	5 and 4	40	0	0	+	+	+	+	+	+
AT-5	4 and 3	40	0	0	+	+	+	+	+	+
AT-6	2 and 3	28	50	0	+	+	++++	+++	++++	+++
AT-7	2 and 2	28	0	0	+	+	+	+	+	+
AT-8	2 and 2	45	50	0	+	+	+	+	+	+
AT-9	2 and 2	45	50	50	+	+	+	+	+	+
AT-10	2 and 2	28	0	0	+	+	+	+	+	+
AT-11	2 and 2	45	0	0	+	+	+	+	+	+
INH	18 (9 groups of 2)	28	0	0	+	+	+	+	+	+
Control, no drug	35	45	95		++	++	++	++	++	++

[a] Dosage of drug diet: (a) = 0.2% daily intake of the drug by the animal weighing 100 Gm. each was estimated to be 150 mg./Kg.; (b) = 0.5% daily intake of the drug by the animal weighing 100 Gm. each was estimated to be 350 mg./Kg. [b] + = Presence of infection with enlargement of gland; − = absence of infection and no enlargement of gland. [c] Per cent drug dosage in diet was 0.2 and 0.5, respectively.

experimental samples on inhibition of *Mycobacterium* after 3 weeks.

In Vivo Test.—This test includes chronic toxicity study on albino rats of our own breed which were infected with *M. tuberculi* and subsequently took a regularly known amount of drug incorporated with their daily food intake. Both low and high doses of individual compound were administered, and the maximum period of observation was 45 days. Within this period, the therapeutic efficacy of the test compounds was studied outwardly by observing the symptoms, particularly the degree of sickness and also the degree of protection of the test animals from infection.

For investigating the effect of prolonged administration of compounds and also for examining the condition of internal organs (7–15)—particularly liver, spleen, and lungs—the duration of oral medication of certain compounds was limited to 28 days, while for others it continued to a maximum period of 45 days. During this particular period, some experimental animals died or were ultimately sacrificed for examination of internal organs. Percentage of mortality was observed to a maximum period of 45 days.

A thorough examination of internal organs of all the test animals receiving different compounds at different dose levels and time periods was conducted.

The percentage of mortality was different with individual test compounds. The therapeutic efficacy was judged by examination of the above-mentioned glands. With some compounds, the degrees of protection from infection were marked—revealed by absence of infection and nonenlargement of glands compared to other test compounds which were relatively less efficacious.

Control animals were infected with *M. tuberculi* only and no drug. They showed the maximum mortality; the glands were highly enlarged compared with all other groups receiving different test compounds. This suggests that the test compounds had definite action in protecting the infected animals from death within a certain period and also in preventing the enlargement of glands to a certain extent.

Table IV indicates the acute toxicity of test compounds, and Table V records the *in vivo* test with varied doses and changes in internal organs.

DISCUSSION

Out of seven compounds of the isonicotinoyl hydrazone series and four compounds of cyanoacetyl hydrazone series, two compounds of each series, showed some antitubercular activity. The compound AT–4 having *p*-dimethylamino group and compound AT–3 having *p*-hydroxy group as substitutions in the benzylidene ring attached to the N^2 atoms of INH molecule showed antitubercular activity comparable to that of INH. The therapeutic efficacy comparable almost to INH was observed in the compound AT–10 with *p*-hydroxy group and in the compound AT–11 having *p*-hydroxy-*m*-methoxy group in the benzylidene ring attached to the N^2 atoms of cyanoacetic acid hydrazide molecule. It suggests, therefore, that the *p*-hydroxy group in the benzylidene ring might have some antitubercular property.

SUMMARY

A series of 11 compounds was synthesized and studied for their antitubercular activity *in vivo* and *in vitro*. Therapeutic activity on sick animals and their subjective improvements and the damages of major internal organs were studied. Acute and chronic toxicities were also noted.

REFERENCES

(1) Hughes, H. B., Biehl, J. P., Jones, A. P., and Schmidt, L. H., *Am. Rev. Tuberc. Pulmonary Diseases*, **70**, 266(1954).
(2) Fox, H. H., and Gibas, J. T., *J. Org. Chem.*, **20**, 60 (1955).
(3) *Ibid.*, **18**, 983(1953).
(4) Valdecasas, F. G., Salva, J. A., and Muset, P. P., *Med. Clin. (Barcelona)*, **4**, 275(1952).
(5) Shchukina, M. N., Pershin, G. N., Makeeva, O. O., Sazonova, E. D., Nikitskaya, E. S., Yanina, A. D., and Yakovleva, A. I., *Doklady Akad. Nauk SSSR*, **84**, 981(1952).
(6) Sah, P. T., and Peoples, S. A, THIS JOURNAL, **43**, 513(1954).
(7) Youmans, G. P., *Ann. N. Y. Acad. Sci.*, **52**, 662(1949).
(8) Doub, L., and Youmans, G. P., *Am. Rev. Tuberc. Pulmonary Diseases*, **61**, 407(1950).
(9) Donvick, R., *Ann. N. Y. Acad. Sci.*, **52**, 671(1949).
(10) Baker, M. J., Schlosser, M. E., and White, H. J., *ibid.*, **52**, 678(1949).
(11) Litchfield, J. T., *J. Pharmacol. Exptl. Therap.*, **97**, 399 (1949).
(12) McKee, C. M., Rake, G., Donovick, R., and Jambor. W. P., *Am. Rev. Tuberc. Pulmonary Diseases*, **60**, 90, 109 21(1949).
(13) Chin, Y. C., Anderson H. H., Alderlon, G., and Lewis, J. C., *Proc. Soc. Exptl. Biol. Med.*, **70**, 158(1949).
(14) Croshaw, B., and Dickinson, L., *Brit. J. Pharmacol.*, **5**, 178(1950).
(15) Haggarth, E., and Martin, A. R., *ibid.*, **3**, 146, 160 (1948); **4**, 249(1949); **5**, 188(1950).