

## SHORT COMMUNICATIONS

PREPARATION OF  
MONOCHLOROACETIC ACID

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Monochloroacetic acid is one of the major chemicals required for the preparation of sodium carboxymethylcellulose. For complete carboxylation (trisubstitution), about 1.75 times the weight of cellulose is required. The authors therefore explored the easiness with which this chemical could be prepared in bulk quantities.

It is often stated in standard texts<sup>1</sup> that monochloroacetic acid can be easily prepared by chlorination of acetic acid in presence of catalysts such as iodine, sulphur, or red phosphorus. On preliminary attempts this could not be at once confirmed. The yields were not satisfactory. A survey of literature showed that even recently research work was undertaken to establish catalytic

chlorination of acetic acid. Mention may be made of a process<sup>2</sup> in which sulphur dioxide and chlorine are simultaneously introduced into acetic acid containing a little active carbon, camphor, pyridine or quiniline etc., which were believed to catalyze the formation of  $\text{SO}_2\text{Cl}_2$ . Irradiation of mixtures of acetic acid and acetic anhydride prior to chlorination with sulphur dioxide and chlorine has also been tried.<sup>3</sup> It was therefore considered necessary to chlorinate acetic acid in presence of different catalysts that have been tried from time to time and find out the best, under the same conditions of experiments. It was found that red phosphorus gave the highest yields, but special care has to be taken in preparing it, or the yields are lower.

Experiments were carried out in a three-necked flask (one litre) fitted with reflux condenser and heated on an oil bath. After the chlorination, the product was distilled, collecting the fraction between 160-190°C.; on re-distillation of this and collecting the fraction between 180-190°C., solid pure monochloroacetic acid crystals were obtained on cooling. Results are presented in Table 1.

TABLE 1.—PREPARATION OF MONOCHLOROACETIC ACID WITH DIFFERENT CATALYSTS.  
ACETIC ACID TAKEN = 100 g.

Chlorinating agent used	Catalyst used	Temp. of reaction (°C.)	Duration of chlorination (hrs.)	Yield of monochloroacetic acid	
				(g.)	% of theoretical
Dry chlorine gas	.. Sulphur powder (4 g.)	118	15	30.0	19.0
Gaseous $\text{SO}_2$ and $\text{Cl}_2$	.. Ultracarbon (1 g.)	115-120	15	18.5	11.7
Gaseous $\text{SO}_2$ and $\text{Cl}_2$	.. Pyridine (1 ml.)	115	15	20.2	12.8
Dry chlorine, $\text{SO}_2$ & 5 g. $\text{Ac}_2\text{O}$	.. Camphor (1 g.)	116-120	20	57.7	36.0
Dry chlorine gas	.. Red phosphorus (4 g. untreated)	105-110	8	25.0	15.9
..	.. Activated red phosphorus (4 g.) with cold water wash	..	8	40.0	25.4
..	.. Activated red phosphorus (4 g.) with hot water wash	..	8	104.0	66.0
..	.. "	..	6	80.0	50.8
..	.. "	..	4	55.0	34.9
..	.. "	..	2	20.0	12.7
..	.. "	..	10	105.0	66.7
..	.. "	..	12	106.0	67.3

[Theoretical yield of monochloroacetic acid=157.5 g.]



All the experiments were carried out under identical conditions (unless otherwise stated), except that the catalyst was varied in the different experiments. With sulphur, carbon, pyridine etc., as catalyst, the yield was very poor (12-20%). When camphor was used in conjunction with sulphur dioxide and acetic anhydride, the yield increased to 36%. Red phosphorus was obtained in the laboratory as a dark red powder. With this as the catalyst, without any pre-treatment, the yield of chloroacetic acid was poor, being only 16%. When phosphorus was boiled with ten times its weight of distilled water (in a beaker), immediately before its use in chlorination, the yield improved (25%). In these experiments, after boiling, the phosphorus was washed twice with *cold* water; but if the washing was done with *hot* water, the yield increased enormously, to 66% of theoretical, corresponding to 104% of the weight of acetic acid used. This experiment was carried out for eight hours. Under the same conditions of treatment of phosphorus, several other experiments were carried out for different periods of time (Table 1). It appeared that the reaction was slower in the beginning but accelerated after about two hours; the reaction proceeded for eight hours, after which there was no further appreciable increase of the yield. Thus, eight-hour chlorination was taken to be the optimum. It is believed that the method can be used for preparation of chloroacetic acid in bulk quantities in the laboratory. We have successfully employed this for preparing our requirements of monochloroacetic acid for carboxymethylation of jute cellulose. It is also believed that the method may be developed for commercial production of monochloroacetic acid.

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## CHEMICAL EXAMINATION OF SNAKE SLOUGHS

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In the indigenous system of medicine and in folklore, a variety of curative properties are attributed to the shed-skin (sloughs) of snakes, particularly to that of Cobra. More recently, studies carried out on the constituents of snake sloughs by Japanese workers have indicated that their ether-soluble component possesses marked anti-carcinogenic activity.<sup>1</sup> In the light of these facts, it was considered of interest to undertake a systematic study of snake sloughs. There was added interest in such a study in order to gain insight in the biological function of the excretory process of moulting in snakes, which has often been associated with their longevity. The present paper describes some of the analytical findings on Cobra shed-skin.

The lipid soluble portion of the skin (4.6%) was found to contain 3.45 percent of cholesterol and 1.4 percent of another sterol which appeared to be an isomer of cholesterol. The presence of such a high percentage of cholesterol in the fatty component of the shed-skin resulting from the excretory process, appears to be significant when contrasted with the cholesterol content recorded in literature for the following oils and fatty materials:

	Percentage Cholesterol
Alligator liver oil ..	0.9 - 1.0
Fish oil (Japanese) ..	0.3
Cod liver oil ..	0.42 - 0.54
Sardine oil ..	0.3 - 1

After saponification, the saturated and unsaturated fatty acids of the oil, obtained from shed-skin, were separated by Twitchell's modified lead-salt method and were found to be 67 percent and 33 percent, respectively. Their physical constants have been determined.

A study of the amino-acid constituents of the hydrolysates of proteinous matter from the shed and live-skins of Cobra, has further shown that the cystein and cystine content is significantly higher in the shed-skin, i.e. 23.7 percent as against 14.7 percent in the live-skin. Furthermore, the tyrosine



content in the shed-skin has also been found to be much higher, about three times that of the live-skin, and in sharp contrast to these values, alanine content of the hydrolysate of the defatted shed-skin is only one-third of that present in the live-skin hydrolysate.

Inorganic matter is present to the extent of 0.7 percent on the weight of the shed-skin and contains 19.76 percent of potassium as against 25.06 percent of sodium, which makes for a significantly high proportion of potassium, for instance, with reference to the sodium-potassium balance recorded for human sweat (0.35 percent sodium and 0.01–0.02 percent potassium).

### Experimental

748 g. of the shed-skin of Cobra, collected from the neighbourhood of Karachi, was exhaustively Soxhleted with ether and the thick fatty residue (134.54 g.) obtained on removal of the solvent, was extracted with boiling acetone. On removal of the solvent, the filtered acetone solution yielded 27.28 g. of a cream-coloured thickish residue (3.6 percent on the weight of the skin). The acetone-insoluble portion (6.71 g.) was an almost colourless, sticky mass which was unsaponifiable and free from nitrogen and phosphorus.

The acetone-soluble fatty matter was saponified by refluxing with 15 percent alcoholic potassium hydroxide and, after removal of alcohol, the unsaponifiable portion was extracted out with ether. The ethereal extract was dried with sodium sulphate, filtered and the solvent removed. The unsaponifiable matter thus obtained (8.85 g.) was chromatographed on neutral alumina (Merck). The first eluate with pet-ether gave a colourless oil (1.6 g.) that analysed for  $C_{27}H_{48}O$ , and the subsequent elutions with benzene and chloroform afforded a glassy residue (2.97 g.) and a solid (2.51 g.), respectively. The former could not be crystallized through any of the common organic solvents, whereas the latter yielded colourless needles, which, on further recrystallisations from methanol, finally showed m.p. 147.5–148°C.,  $[\alpha]_D^{25} -34.5^\circ$  (c.1,  $CHCl_3$ ). It formed a crystalline acetate, m.p. 114.5°C., on reacting with acetic anhydride in presence of pyridine at room temperature and responded to Liebermann-Burchard test. It analysed for  $C_{27}H_{46}O$  (Found: C, 83.69; H, 11.46 percent;  $C_{27}H_{46}O$  requires C, 83.93; H 11.91 percent), and, on admixture with an authentic specimen of cholesterol, did not show any depression in melting point.

The methanolic mother-liquor of cholesterol yielded another sterol, m.p. 135–37°C.,  $[\alpha]_D^{18} -18^\circ$  (c.1,  $CHCl_3$ ) (Found C, 84.10; H, 12.18 percent;  $C_{27}H_{46}O$  requires C, 83.93; H, 11.91 percent). This compound also responded to Liebermann-Burchard test, formed a digitonide, and with acetic anhydride in presence of pyridine, gave a crystalline acetate, m.p. 103–4°C.

The total sterol, estimated as digitonide, was found to be 22 percent of the unsaponifiable.

The water-soluble portion of the saponified fatty matter containing the potassium salt of fatty acids, was worked up according to the usual procedure and the total fatty acids obtained (15.56 g.) were separated into saturated (10.5 g.) and unsaturated (4.8 g.) portions by using Twitchell's modified lead-salt method. The neutralization equivalent, iodine value and the mean molecular weight of the saturated acids were found to be 237, 10.02 and 236.5, respectively. In case of unsaturated acids, the values were 201.6, 61.63 and 278.

### Estimation of Inorganic Matter

After extraction with ether, the residual skin was extracted with 30 percent ethanol at room temperature. The extract, on removal of solvent, yielded a semi-solid mass (2.1 percent on the weight of shed-skin). A portion of the residue was ashed in a platinum crucible and the constituents of the ash (0.7 percent on the weight of the skin) were determined. Sodium, potassium, chlorine and phosphorus (present as phosphate) were found to be 25.06, 19.76, 10.0 and 0.26 percent, respectively.

### Protein Analyses of Shed and Live-skins

(i) *Shed-skin*.—After extraction of the shed-skin with ether and aqueous alcohol, as mentioned above, the residual skin was hydrolysed by heating with 6N hydrochloric acid at 100°C. in a sealed tube for 24 hours.

Practically, the whole of the skin was hydrolysed, leaving a negligible residue that was filtered off. The various amino-acid components of the hydrolysate were identified by two dimensional paper chromatography using BuOH(100)/AcOH(22)/H<sub>2</sub>O(50) (1st dimension) and Phenol (100)/H<sub>2</sub>O(39) (2nd dimension) as the two solvent mixtures and ninhydrin as spraying agent. The estimation of different amino-acids in the protein hydrolysate was done by ninhydrin colorimetric method.<sup>2</sup>



(ii) *Live-skin*.—The live-skin was carefully dissected out from a freshly killed Cobra and was freed from other tissues. It was then passed through grades, dipped into xylol, immersed in molten paraffin and heated in an oven for two hours. It was again dipped into xylol and finally heated in the oven for another half an hour. Following these operations, the dehydrated skin was hydrolysed and the different amino-acids in the hydrolysate were determined quantitatively according to the procedure followed in (i). The comparative data for the amino-acid constituents in the hydrolysates of the shed and live-skins are given in Table 1.

TABLE I.

Amino-acids	Percentage amount in shed-skin	Percentage amount in live-skin
1. Leucine and Isoleucine ..	3.5	2.0
2. Valine ..	4.5	3.6
3. Tyrosine ..	0.9	0.35
4. Alanine ..	2.5	7.0
5. Threonine ..	10.4	10.0
6. Arginine ..	12.5	5.25
7. Lysine ..	6.6	14.75
8. Cystine and Cystein ..	23.75	14.75
9. Aspartic Acid	2.5	2.5
10. Glutamic Acid	13.9	10.4
11. Serine ..	13.3	9.15
12. Proline ..	3.0	5.85
13. Phenyl alanine	in traces	in traces

Further work on problems arising out of the present study is in progress.

**Acknowledgement.**—The authors wish to record their gratitude to Dr. Salimuzzaman Siddiqui for his advice and suggestions. Thanks are also due to the micro-analytical section for the analysis of inorganic matter.

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### ISOLATION OF CLERODOLONE, CLERODONE, CLERODOL AND CLEROSTEROL FROM CLERODENDRON INFORTUNATI (BHAT)

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The stem and leaves of the plant *Clerodendron infortunati* of Indian origin have been quite extensively investigated. Barton and co-workers have given the structure of the isolated compounds.<sup>1,2</sup> No chemical investigation of the root materials so far appears in the chemical literature.

The present investigation carried out with the plant roots from East Pakistan have given four new, hitherto unreported compounds. The alcoholic extractive of the fresh root materials was concentrated *in vacuo* and the greenish gummy solid precipitated from the mainly aqueous phase (A) was leached out with petroleum ether. The residue on repeated crystallisations gave colourless crystalline *clerodolone*, m.p. 299-300°,  $\alpha_D^{25} + 8^\circ$  (Found: C, 77.85; H, 10.72; O, 11.45%, Mol. wt. (Rast) 414.  $C_{27}H_{44}O_3$  requires C, 77.83; H, 10.65; O, 11.52%; Mol. wt. 416.6). It had  $\lambda$  max 202 m $\mu$  ( $\epsilon$ 7,800) in ethanol and  $\nu$  max (KBr) 3400, 3300, 3055, 1700, 1690, 1645, 885 cm<sup>-1</sup>. Clerodolone absorbed 0.85 moles of hydrogen on microhydrogenation over platinum catalyst in glacial acetic acid.

The petroleum ether soluble fractions on chromatography over neutral alumina gave a small quantity of colourless needles of *clerodone*, m.p. 260° from the benzene eluate (Found: C, 82.04; H, 11.02; O, 7.54% M. wt. (Rast) 390.  $C_{29}H_{46}O_2$  requires C, 81.63; H, 10.87; O, 7.50%; M. wt. 426.7). It had  $\lambda$  max 205 m $\mu$  ( $\epsilon$ 3,500) in ethanol and  $\nu$  max (KBr) 1700 and 1670 cm<sup>-1</sup>. On microhydrogenation it absorbed 1.0 mole of hydrogen in glacial acetic acid over platinum catalyst. The benzene-ether (1:1) eluate first gave a solid which on crystallisation from petroleum ether (60-80°) gave *clerodol*, m.p. 205°,  $\alpha_D^{25} + 36^\circ$  (Found: C, 82.25; H, 11.3; O, 7.4%. Mol. wt. (Rast) 421.  $C_{30}H_{48}O_2$  requires C, 81.8; H, 11.0; O, 7.37%; Mol. wt. 440.7). It had  $\lambda$  max 203.5 m $\mu$  ( $\epsilon$ 8,800) in ethanol and  $\nu$  max (KBr) 3400, 1640 and 885 cm<sup>-1</sup>. Clerodol absorbed 0.9 moles of hydrogen when microhydrogenated over platinum catalyst in glacial acetic acid.



Further elution with the same solvent gave *clerosterol*, m.p.  $148^{\circ}$ ,  $\alpha_D^{25} - 31^{\circ}$  (Found: C, 81.43; H, 11.11; O, 7.22%; Mol. wt. (Rast) 341.  $C_{28}H_{46}O_2$  requires C, 81.10; H, 11.18; O, 7.72%. Mol. wt. 390.6). It had  $\lambda_{max}$  203.6  $m\mu$  ( $\epsilon$ 9,700) in ethanol and  $\nu_{max}$  (KBr) 3400, 3300, 1640, 885  $cm^{-1}$ .

On paper chromatography of aqueous mother liquor (A), seven sugars raffinose, lactose, mathose, sucrose, galactose, glucose and fructose could be identified when run against known standards raffinose, lactose, maltose, sorbose, arabinose, fructose, xylose, rhamnose and ribose.

A more detailed report of this work is being published separately.

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## RESIDUAL ACTION OF MAKROLIN AS COMPARED WITH OTHER INSECTICIDES ON TRIBOLIUM CASTANEUM (HERBST) TENEBRIONIDAE: COLEOPTERA

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### Introduction

The evaluation of residual effects of insecticides is of great practical importance before recommending them for the control of insects. A variety of environmental factors influence the residual toxicity of insecticides, but the loss of residual action of insecticides owing to physical factors have been noted by several workers. Sweetman<sup>1</sup> reported that a temperature of  $32^{\circ}C$ . with high humidities decreased the residual toxicity of DDT. Teotia and Dahm<sup>2</sup> found that a high temperature ( $108 \pm 2^{\circ}F$ .) with a low humidity ( $36 \pm 5\%$  R.H.) shortened the residual action of a number of insecticides more than a low temperature ( $52 \pm 2^{\circ}F$ .)

with high humidity ( $76 \pm 5\%$  R.H.) Kalkat, Davidson and Bross<sup>3</sup> reported that at  $90^{\circ}F$ . and 55% R.H. the residual action of Aldrin, Heptachlor, Heptachlor epoxide and Diazinon was 8, 10, 28 and 99 days, respectively, whereas at the same relative humidity with  $110^{\circ}F$ . the residual action was 4, 4, 21 and 28 days, respectively.

The present studies were undertaken to evaluate the residual action of the newly developed insecticide Makrolin at  $98 \pm 2^{\circ}F$ . and  $75 \pm 5\%$  R.H. in comparison with other organic insecticides such as Aldrin, Dieldrin, Endrin, Methoxychlor, Lindane, Heptachlor and Pyrethrum. Their relative toxicities were bioassayed by using the red flour beetle, *Tribolium castaneum* (Herbst), as a test insect.

### Materials and Methods

For rearing the red flour beetle, 0.5 lb. of wheat flour and a table spoonful of brewer's yeast were put in a beaker of two litres capacity. Approximately 200 adults, about four weeks old, were liberated in the beaker. After one week the adults were sifted out with a twenty-mesh sieve, and they were discarded. The eggs and recently hatched larvae, which passed out through the sieve, were used to get adults of known age. Under laboratory conditions, it took nearly five weeks for the red flour beetle to develop from eggs to adults. The breeding receptacles were prepared weekly. This provided enough beetles of known age for testing throughout the experimentation.

Whatman's filter paper of 6 cm. diameter was used as surface for preparing the residue film of an insecticide. Films of different insecticides were made by using 1 ml. of 2 percent solution of each insecticide prepared in acetone. The papers were kept at the room temperature for drying for 24 hours before using them for experimentation; the final amount of residue on each paper was 0.714  $mg/cm^2$ . The beetles were exposed to the treated surface for three hours in all cases. After the exposure the beetles were gently transferred on to an untreated filter paper placed in an already marked petri-dish. Against the film of each insecticide 25 beetles were exposed with five replications. A temperature of  $98 \pm 2^{\circ}F$ . and a relative humidity of  $75 \pm 5$  percent were used for ascertaining their effects on the residual duration of Makrolin and other insecticides. The humidity was controlled by using 75 g. of potassium hydroxide per 100 ml. water at the bottom of dessicators, kept in a temperature controlled incubator. The mortality percentage was noted



after 48 hours, and it was corrected by using the Abbot's formula. After using the treated filter papers for experimentation, they were returned to the dessicator kept at  $98 \pm 2^\circ\text{F}$ . and  $75 \pm 5\%$  R.H. The experiments were discontinued after 93 days.

### Results and Discussion

Results obtained are shown in Figs. 1 and 2. The residues of Aldrin and Dieldrin were effective up to 93 days, giving 12 and 24 per cent mortality,

respectively, of red flour beetles. Lindane, Heptachlor and Endrin residue films lost their toxicity within 75, 65 and 62 days, respectively. On the 60th day Lindane, Heptachlor and Endrin residue gave mortality of 52 percent, 40 percent and 32 percent, respectively. Methoxychlor, Pyrethrum and Makrolin had lasting effect of 10, 8 and 7 days, respectively.

The present results show that at  $98 \pm 2^\circ\text{F}$ . and  $75 \pm 5\%$  R.H. the residual effects of Aldrin, Dieldrin, Heptachlor and Lindane were much

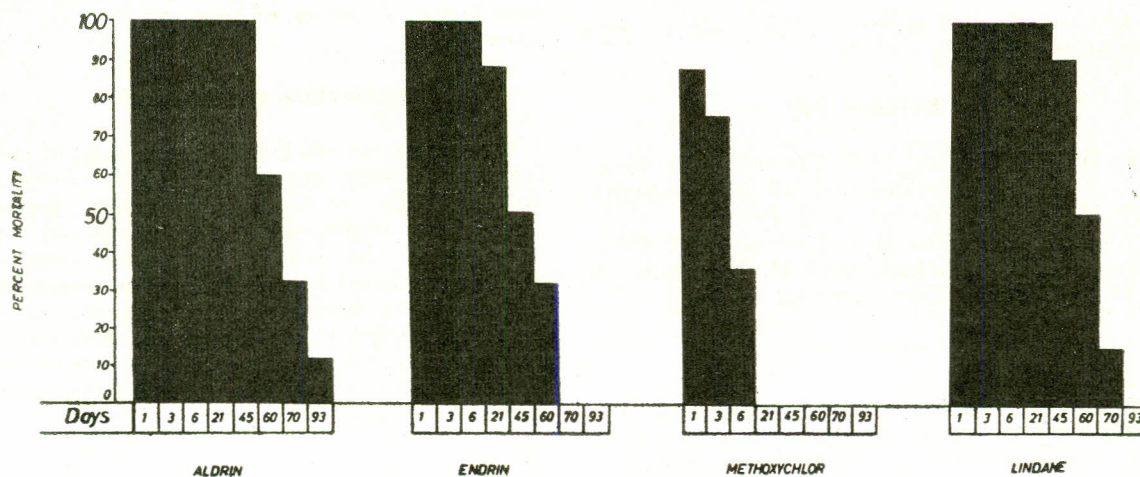


Fig. 1.—Residual Life of Aldrin, Endrin, Methoxychlor and Lindane at Temperature  $98 \pm 2^\circ\text{F}$ . and R.H.  $75 \pm 5\%$ .

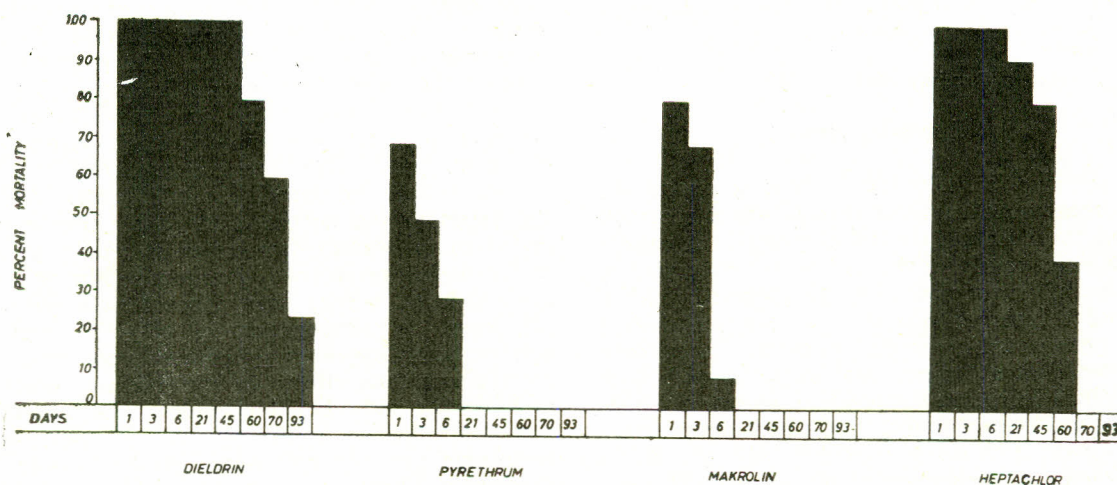


Fig. 2.—Residual Life of Dieldrin, Pyrethrum, Makrolin and Heptachlor at Temperature  $98 \pm 2^\circ\text{F}$ . and R.H.  $75 \pm 5\%$ .



longer than those of Makrolin, Pyrethrum and Methoxychlor.

According to Kalkat *et al.*<sup>3</sup> the residual effect of Aldrin and Heptachlor was decreased when tested against red flour beetles at a high temperature of 90°F. and low humidity of 55±5% R.H. But their lasting effect was increased at a temperature of 98±2°F. and a higher humidity of 75±5% R.H. This shows that the increase in humidity at higher temperature enhanced the residual action of Aldrin and Heptachlor.

Teotia and Dahm<sup>2</sup> reported that a temperature of 52±2°F. and 75±5% R.H. by using houseflies as test insects, the residue of Dieldrin gave 100 percent kill on the 65th day and in the case of Lindane and Aldrin the residual effects persisted up to 65 days. Similar effects were found in the case of Dieldrin, Aldrin and Lindane at 98±2°F., and 75±5% R.H. against red flour beetles. Dieldrin gave high mortality up to 70 days, while a decline in the toxicities of Aldrin, Lindane and Heptachlor was evident on the 60th day.

The present investigation showed that a high temperature of 98±2°F. and a high humidity of 75±5% increased the residual action of Aldrin, Dieldrin, Lindane, Heptachlor and Endrin, while it was shortened in the case of Methoxychlor, Pyrethrum and Makrolin. In view of the fact that the residual effect of Makrolin has been found to be of short duration in the regions of high temperature and high humidity, it may be possible to use it without any health hazard in controlling pests on crops, vegetables and fruits when sprayed about eight to ten days before harvesting.

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## ANTIBIOTIC-PRODUCING MICRO-ORGANISMS FROM WEST PAKISTAN SOILS

### Part II.—*Aspergillus Quadrilineatus*-Some Bio-Chemical Studies

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### Introduction

The isolation of *Aspergillus quadrilineatus* which was shown to elaborate three different active materials at different stages of its growth has been reported earlier.<sup>1</sup> These active materials are probably penicillin, quadrilineatin, nidulin and *nor*-nidulin. Further studies were therefore carried out on their extraction, fractionation by column chromatography, and estimation their of antibacterial activity. The antibacterial activity of the culture broth was assayed in terms of units of penicillin/ml. by using the method of Heatly,<sup>2</sup> with slight modification in which cylinders were replaced by thick sterilized filter paper discs. The antibacterial activity of the metabolic solution was determined from a standard curve against the zone of inhibition (in mm.).

### Experimental and Results

*Original Broth.*—The antibacterial activity of the broth obtained from 4,8,12,16,24 and 32 days old cultures grown at pH 5,6 and 7 in czapek-Dox medium, has been determined and the results are reported in Table 1. Optimum yields are obtained at pH 7 in 16 days old cultures.

*Extract.*—The broth was cooled to 5°C., acidified with hydrochloric acid to pH 2.0, and active material was extracted with two volumes of ether. The ethereal layer was treated quickly with 1/20 of its volume of M/50 phosphate buffer at pH 7 into which the major portion passed. The cooled buffer was then in turn acidified to pH 2 and extracted with two volumes of ether from which the active material was again extracted with 0.1N sodium carbonate solution in a volume 1/100 that of the original broth and pH adjusted. The antibacterial activity of the original broth and that of the extract with M/50 phosphate buffer and 0.1N sodium carbonate was assayed<sup>3</sup> and the results are reported in Table 2.



TABLE 1.—ANTIBACTERIAL ACTIVITY IN TERMS OF UNITS OF PENICILLIN/ML. IN ORIGINAL BROTH.

Age in days	4	8	12	16	24	32
pH 5	—	—	2.0	2.4	2.1	1.8
pH 6	—	—	2.4	3.8	2.2	1.8
pH 7	—	1.8	2.4	4.8	2.2	1.8

TABLE 2.—ASSAY OF ANTIBACTERIAL MATERIAL IN CULTURE EXTRACTS IN TERMS OF PENICILLIN UNITS.

Age in days	4	8	12	16	24	32
Original Broth	..	..	2.4	4.2	3.8	2.4
M/50 Phosphate Buffer	..	..	16.0	36.0	28.0	14.0
0.1 N Sodium Carbonate	..	..	110.0	190.0	170.0	100.0

*Chromatographic Separation.*—The active broth was subjected to column chromatography using alumina as an adsorbent.<sup>4</sup> (alumina was prepared by washing with dilute hydrochloric acid and then dried at 110°C.). Chloroform extract of the broth was percolated through the packed column and then eluted with phosphate buffer. The elute was collected in twenty-five different fractions of 2 ml. each. Each of these fractions was tested for antibacterial activity using *S. aureus* as the test organism by the method of Vincent and Vincent.<sup>5</sup> The experiment was repeated with broth obtained from 12,16,24 and 32 days old cultures. The active materials are indicated to concentrate in three bands (Table 3). The fractions of last band from 24 and 32 days old cultures are also active against *M. tuberculosis*<sup>6</sup>

TABLE 3.—COLUMN CHROMATOGRAPHY OF CULTURE BROTH EXTRACT (FORMATION OF ACTIVE ZONES).

Age in days	12	16	24	32
Tube No.				
1	—	—	—	—
2	—	—	—	—
3	—	—	—	—
4	—	—	—	—
5	+	+	—	+
6	+	+	—	+
7	+	+	+	+
8	—	+	+	+
9	—	+	+	+
10	—	—	+	—
11	—	—	—	—
12	—	—	—	—
13	—	—	—	—
14	—	—	+	+
15	+	+	+	+
16	+	+	+	+
17	+	+	+	—
18	—	—	+	—
19	—	—	+	—
20	—	—	—	—
21	—	—	+	+
22	—	—	+	+
23	+	+	+	+
24	+	+	+	—
25	+	+	—	—

— denotes Inactivity; + denotes Activity.

possibly indicating nidulin and *nor*-nidulin. Quadrilineation was not found to be absorbed on the column.

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