SHORT COMMUNICATIONS

STUDIES ON FAGONIA CRETICA LINN. PART II

WASIF HUSSAIN, S.F. HUSSAIN, M. IKRAM AND S.A. WARSI

North Regional Laboratories, Pakistan Council of Scientific and Industrial Research, Peshawar

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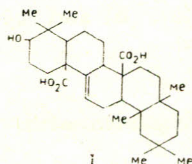
Introduction

In continuation of the study of the constituents of *Fagonia cretica*, acetone insoluble fraction of the alcoholic extracts have now been examined. As reported earlier,[1] a glycosidic bitter complex as a light cream coloured powder was obtained which yielded a crystalline product on acid hydrolysis. The crystalline product has now been identified as chinovic acid (I). This acid has been reported[2] to be present in cinchona bark as the glycoside, chinovin. Since this acid is also obtained from *F. cretica* on acid hydrolysis of the extract, it is suggested that chinovic acid is also present in this plant in the form of a glycoside, although we could not isolate the glycoside in the pure form. The medicinal properties of the cinchona bark have been reported[3] to be fabrifuge, tonic and astringent. Similar properties have been reported[4] for *Fagonia cretica*. The glycosides of the two plants being derivatives of the same aglycone, chinovic acid, may explain the common curative properties.



Alcoholic extract of the dry plant after complete removal of the solvent was exhaustively extracted with acetone. In an attempt to isolate the glycoside, the bitter acetone insoluble fraction was dissolved in methanol and treated with 10% methanolic sodium hydroxide. The greenish precipitate thus obtained was centrifuged, washed with ethanol and for further purification redissolved in methanol containing 5% HCl and reprecipitated by the addition of methanolic sodium hydroxide. The yellowish powder obtained on drying the precipitate yielded 0.095% chinovic acid on acid hydrolysis. However, when the entire crude acetone insoluble fraction was directly subjected to acid hydrolysis, the yield of chinovic acid was found to be 0.68% by weight of the dry material. The aglycone acid was therefore, isolated by acid hydrolysis of the crude acetone insoluble fraction. Attempts to isolate the glycoside, however, have not been successful.

Methyl and acetyl derivatives of chinovic acid were prepared in order to compare with the known acid. Methylation of acetyl chinovic acid was also carried out and monoacetyl dimethyl ester of chinovic acid was obtained. Analysis of this compound was not considered necessary as the I.R. Spectrum confirmed the presence of only the ester group in the molecule (νmax. 1725 cm^{-1}) and the absence of -OH indicating that both the carboxyl groups have been methylated.

Experimental

Melting points are uncorrected. Analyses were carried out by A. Bernhardt, Max Planck Institute, Ruhr, West Germany. Infra-red Spectra were determined as Nujol mulls using Beckman IR-5.

Shade-dried plants (5 kg.) were chopped and percolated with ethanol. The solvent was removed under vacuum. The residue after removing the solvent was extracted with acetone and the insoluble fraction (200 g.) thus obtained was subjected to acid hydrolysis.

Isolation of Chinovic Acid.—The acetone insoluble fraction (35 g.) was refluxed for one hour on a water bath. The volume was reduced to half and the contents allowed to cool when crystals, settled down. The crystalline residue was filtered under suction, washed and dried (6.0 g; 0.68%). Several recrystallisations from ethanol-water yielded colourless crystals, m.p. 305-7° (lit; 298°)[2] (Found C, 74.14; H, 9.64; O, 16.62; Calculated for $C_{30}H_{46}O_5$, C, 74.03; H, 9.53; O, 16.45). νmax. 3500, 1665 cm^{-1}. It gave a positive Lieberman test. The acid was found to be insoluble in all solvents except hot ethanol.

Acetylation of Chinovic Acid.—Chinovic acid (0.43 g.) mixed with acetic anhydride (25 ml.) and eight drops of pyridine was refluxed for two hours at 115-120°C. on an oil-bath. A light yellow solution was obtained which on concentration yielded colourless crystals (0.41 g.). Recrystallised from dilute alcohol, m.p. 285°, (lit. 284°)[2]. (Found C, 72.67; H, 9.27; O, 18.25; CH_3CO, 8.83. Calculated for $C_{32}H_{48}O_6$, C, 72.69; H, 9.15; O, 18.16: 1×CH_3CO, 8.17), νmax. 1720, 1665 cm^{-1}.

Methylation of Chinovic Acid.—Chinovic acid (0.4 g.) suspended in 10% aqueous sodium hydroxide (16 ml.) was vigorously stirred using a magnetic stirrer and dimethyl sulphate (2 ml.) slowly added. The stirring was continued for six hours and during this period more dimethyl sulphate (8 ml.) and solid sodium hydroxide (3.0 g.) were added alternately in small portions at half hour intervals. The solid substance which precipitated at the end of the reaction was sucked off, washed thoroughly with water and dried over P_2O_5 under vacuum (0.44 g.). Recrystallisation from acetone-water afforded silken white crystals m.p. 164-6° (lit. 175-6°)[2] (Found; C, 74.66; H, 9.68; O, 15.83; O-CH_3, 11.78. Calculated for $C_{32}H_{50}O_5$, C, 74.67; H, 9.79; O, 15.54; 2×OCH_3, 11.7), νmax. 1700, 1685 cm^{-1}.

Methylation of Acetyl Chinovic Acid.—Acetyl chinovic acid (200 mg.) mixed with acetone (30 ml.), dimethylsulphate (3 ml.) and anhydrous potassium carbonate 4.0 g.) was refluxed on a water bath for 4 hours. The solvent was then completely removed and water (40 ml.) added. The crystals thus obtained were dried over P_2O_5 m.p. 190°, νmax. 1725 cm^{-1}.

Acknowledgement.—The authors are grateful to Dr. G. Macrea, Department of Chemistry, University of Peshawar, for running the IR Spectra.

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EQUIVALENT AND MOLECULAR WEIGHT DETERMINATION OF ALKALOIDS BY MICRO-TITRATIONS IN NON-AQUEOUS SOLVENTS

M. RAFIULLAH AND M. IKRAM

North Regional Laboratories, Pakistan Council of Scientific and Industrial Research, Peshawar

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Introduction

Methods such as ebullioscopic (elevation of the B.P. of a solvent), cryoscopic (depression of the freezing and m.p. of the solvent) etc. are available for the micro-determination of the molecular weight of a substance. The most common method is that of Rast. It is based on the principle that a solute depresses the melting or the freezing point of a solvent proportional to the mole fraction of solute present. It has certain limitations *i.e.*, the solute must be soluble in the solvent, it should not decompose at the temperature of the M.P. of the solvent, it should not react with the solvent. Secondly, it was observed that the molecular weight found by Rast method varies with the amount of solute in the solvent. The results are given in Table 2. Determination of molecular weight by titrating the compounds in non-aqueous solvent which has been used in the present study is comparatively simpler and more general (less limitation) than the Rast method. Hans Brockmann and Ernst Meyer[1] have developed a method for the determination of molecular and equivalent weights for weak acids in a specially designed apparatus by potentiometric titration in non-aqueous solvents. In the present investigation we have determined the molecular weight of various alkaloids by titrating in non-aqueous medium (Table 1(a), (b) and (c) No special apparatus is required.

Experimental

Normality of perchloric acid = 0.1N; Normality of sodium acetate = 0.1N; Mercuric acetate solution = 3% w/v; Indicator = Crystal violet; Weight of alkaloid taken for each titration
=10-20 mg.

(a) *Back Titration.*—10-20 mg. of alkaloid is dissolved in 5 ml. standard 0.1N perchloric acid and titrated against standard sodium acetate.

TABLE 1 (a).

S. No.	Alkaloid	Observed molecular weight	Calculated molecular weight	% error
1.	Atropine ..	293.0	289	1.3
2.	Atropine sulphate ..	700.8	676	3.5
3.	Amphetamine sulphate..	374.0	368.5	1.4
4.	Berbenine[2] ..	298.0	298	0.0
5.	Berbericine[2] ..	334.0	334	0.0
6.	Cocaine ..	295.0	303	2.6
7.	Codeine ..	291.0	299	2.66
8.	Cinchonine hydrochloride	361.0	366.9	1.6
9.	Harmine ..	215.0	212	1.3
10.	Harmaline ..	213.8	214	0.09
11.	Morphine ..	313.0	303.45	3.2
12.	Narcodine ..	390.9	413	5.4
13.	Nicotinamide ..	120.2	122	1.6
14.	Nicotine hydrogen tartarate ..	488.0	498.45	2.2
15.	Papaverine ..	334.0	340	1.7
16.	Procaine hydrochloride	283.0	272	4.0
17.	Tabernaemontanine ..	356.4	356	0.11
18.	Vasicine ..	198.0	188	5.3
19.	Vasicinine[3] ..	207.0	204	1.4

(b) Dissolving the base in 3% mercuric acetate solution and titrating it directly with standard perchloric acid solution.

TABLE 1(b).

S. No.	Alkaloid	Observed molecular weight	Calculated molecular weight	% error
1.	Ephedrine hydrochloride	206.0	201	2.4
2.	Pilocarpine hydrochloride	248.0	244.72	1.3
3.	Pyridoxine hydrochloride	197.0	205	3.9
4.	Yohimbine hydrochloride	394.0	390.92	0.76

(c) *Direct Titration*.—10-20 mg. of alkaloid was dissolved in 5 ml. glacial acetic acid and it was titrated by standard 0.1N perchloric acid solution

TABLE 1(c).

S. No.	Alkaolid	Observed molecular weight	Calculated molecular weight	% error
1.	Canadine	334.0	339.3	1.4
2.	Nicotine	162.0	162	0.0

TABLE 2.—MOLECULAR WEIGHT OF ALKALOIDS BY RAST METHOD.

S. No.	Alkaloid	Ratio of the solute to the solvent (Camphor) 1:10	1:15	1:20	Actual mol. wt.
1.	Atropine ..	350	233	261	289
2.	Codeine ..	285	246	208	317
3.	Colchicine ..	373.9	567	277	399
4.	Narcotine ..	281	391	412	413
5.	Papaverine ..	223	255	227	339
6.	Tabernaemontanine ..	—	—	350	356

Acknowledgement.—The authors wish to thank Dr. S. A. Warsi, Director, North Regional Laboratories, Peshawar, for his keen interest in the work.

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TURPENTINE OIL-BASED CHEMICALS

Part II.—Production of p-Cymene from Pinene-free Turpentine Oil

ABDUL SATTAR, ZAHUR-UD-DIN, M.K. BHATTY AND KARIMULLAH

West Regional Laboratories, Pakistan Council of Scientific and Industrial Research, Lahore

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Introduction

As reported in the earlier communications[1-3] from these Laboratories, indigenous turpentine oil has already been effectively utilized for the production of terpin hydrate and terpineol. However, only the pinene part (about 50%) of the oil is used for the production of these materials. The residual oil consists predominently of Δ^3-carene which is recovered as a by-product. This communication deals with the utilization of the residual oil for the production of p-cymene.

p-Cymene occurs naturally in numerous essential oils and commands wide application in cos-

metic industry. It is used for perfuming soap and in many technical preparations in which it is desirable to suppress unpleasant odours.[4] It is also used in synthetic perfumery and for the production of many valuable chemicals. With strong oxidants, it gives p-toluic acid and ultimately terephthalic acid,[5] while with mild oxidising agents it gives p-cymene hydroperoxide which, in turn, can be converted into p-cresol and acetone.[6]

The conversion of turpentine oil to p-cymene has generally been brought about through catalytic dehydrogenation in the vapour phase at 400-500° in the presence of chromium oxide (5-10%) supported on Al_2O_3 SiO_2[6-8,10-11] etc. The maximum yields of crude p-cymene (along with some m-cymene and menthadienes) from almost pure carenes have been reported to be nearly 45-50%. The conversion of carenes to p-cymene has also been effected in liquid phase[9] by refluxing the former with some catalysts.

In our studies the oxides of some commonly available transition metals as possible catalysts have been tried on pumice stone, partially dehydrated gypsum and fire-brick supports. Conditions have been established whereby 54% of p-cymene can be obtained from the residual turpentine oil, when nickel oxide is used as a catalyst.

Materials

(1) *Pinene-free Turpentine Oil.*—The turpentine oil left after the production of terpin hydrate was freed from the acidic catalyst[2] by washing with water two to three times. The oil was then steam-distilled and the distilled oil dried over anhydrous sodium sulphate. This oil was utilised throughout these investigations.

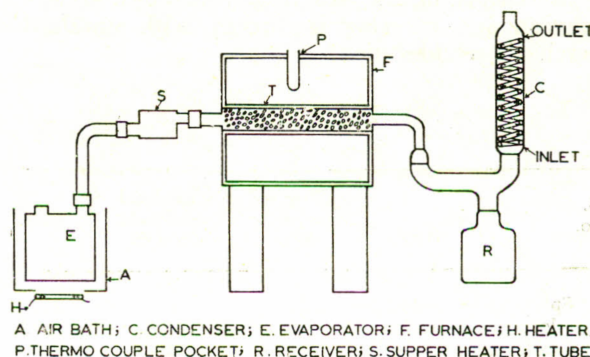
(2) *Inert Supports.*—(a) *Pumice Stones*: 10-20 Mesh material was selected from the stones supplied by the B.D.H. Ltd. These were first washed with dilute sulphuric acid and then thoroughly with water and dried.

(b) *Gypsum*: 10-20 Mesh locally available gypsum was heated to 200° for 2 hours to partially dehydrate it.

(c) *Fire-bricks*: Well-burnt clay bricks were broken into pieces and 10-20 mesh of the material was selected. This material was washed with dilute sulphuric acid and finally with water several times and dried.

(3) *Catalysts.*—The following laboratory grade salts were used as catalysts on the supports: nickel nitrate, cobalt nitrate, ferric nitrate, copper nitrate, ammonium dichromate, ammonium molybdate, ammonium metavanadate.

(4) *Apparatus.*—(Fig. 1) The vapour phase catalytic dehydrogenation was carried out in a stainless steel tube T ($\frac{3}{4}''$ dia, 28″ long) placed in an electric horizontal tube furnace F (2-feet long). The tube length inside the furnace was packed with a catalyst-support system. The temperature of the furnace was controlled with a thermostat. The oil was vapourised in a stainless-steel vessel E having one litre capacity and connected at one end to the reaction tube. The vessel was heated electrically and the heating was regulated with a relay system. By this controlled heating, the rate of flow of the vapours was adjusted. The other end of the reaction tube was connected to a water-cooled condenser C and a receiver R to collect the condensate.



A AIR BATH; C CONDENSER; E. EVAPORATOR; F. FURNACE; H. HEATER; P. THERMO COUPLE POCKET; R. RECEIVER; S. SUPPER HEATER; T. TUBE

Fig. 1.—Apparatus for the production of p-cymene.

Experimental

Such amounts of the catalytic salts as would give 5% and 10% of their oxides on 200 g. of a support, were dissolved in 100 ml. and 200 ml. of distilled water respectively. The solutions were mixed with the supports and the mixtures evaporated to dryness on a steam-bath with continuous stirring. The impregnated materials were then heated in the reaction tube placed in the tube-furnace at 400° for 4 hours while passing a slow stream of dry air through it.

Production of p-cymene.—The tube filled with a catalyst-support system was heated to the required temperature. The pinene-free distilled turpentine oil was vapourised in the vessel E and the vapours were pre-heated to 250° with the superheater S (Fig. 1). The oil vapours were then passed through the catalyst bed. The vapours issuing out of the other end of the tube were condensed and the condensate was collected. The condensate was fractionally distilled into the following four cuts:

(i) b.p. 155° (ii) b.p. 155-173° (iii) b.p. 173-178° and (iv) b.p. 178°.

The fraction (iii) was freed from the unsaturated compounds by shaking it with 80% H_2SO_4 for 2 hours. The unreacted oil was washed with water, then with 10% NaOH solution and finally steam-distilled. The clear oil obtained was a mixture of p-and m-cymenes (Refractive index at 20°= 1.4900, Iodine value 0).

Analytical Methods.—The clear oil was analysed for its constituents by a chemical method [12] as well as by gas-liquid phase chromatography. The gas chromatographic analysis was carried out by using a column filled with celite (30-80 mesh) coated with 5% silicon elastomer E-301 (column temperature 160°; bridge current 150 mA; nitrogen flow rate 1.4L/hour; chart speed 12″/hour; column inlet pressure 68 cm; column outlet pressure 50 cm.).

Discussion and Conclusion

These studies show (Table 1) that among the catalysts so far tried nickel oxide gave a maximum conversion of Δ^3-carne (pinene-free turpentine oil) to p-cymene. Further 5% of the catalysts gave as good a yield as that obtained with 10% of the catalyst.

Fire-bricks appeared to be the best inert support. This support has the qualities of both cheapness and of having a large surface area due to its high porosity.

Variation in the feed rate of turpentine oil (T.O.) vapours showed that the optimum feed rate was 0.50 g. T.O./hour/g. of the catalyst system. With feed rates of 0.45, 0.50, 0.55, 0.60 g. T.O./hour/g. catalyst, the yields were 54%, 54%, 49% and 41% respectively.

The most suitable size of the catalyst material was found to be 10-20 mesh. The yields of p-cymene decreased with an increase in the size of the catalyst obviously, because of the shorter contact time while with finer mesh material the yields were higher but the reaction tube usually clogged up due to polymerisation of the turpentine oil. This polymerisation also deactivated the catalyst.

The optimum temperature range for the production of p-cymene was 450-500° (Table 1).

TABLE 1.—PERCENTAGE YIELD OF P-CYMENE (ON THE BASIS OF PINENE-FREE TURPENTINE OIL).

Percentage of the catalyst = 5%
Flow rate of the vapours = 0.50 gm. of T.O./hr./g. catalyst
Preheating of the vapours to = 250°
Size of the support = 10-20 mesh.

Support	Temp. °C.	% yield with catalyst						
		NiO	Cr_2O_3	MoO_3	CoO	V_2O_5	Fe_2O_3	CuO
Fire-bricks ..	400	44	38	32	28	29	27	27
	450	53	45	38	35	34	33	33
	500	54	48	42	40	38	36	37
Gypsum	400	40	36	30	26	28	25	26
	450	47	42	37	34	33	32	32
	500	51	46	41	37	37	35	35
Pumice stones ..	400	39	35	30	25	26	26	26
	450	46	39	37	32	31	32	31
	500	50	44	41	35	35	36	34

Below this temperature, a large quantity of unsaturated hydrocarbons were formed in the pyrolysate, thereby decreasing the yields of p-cymene, while above this temperature, some cracking of the terpenes started which gave low boiling hydrocarbons like benzene, toluene, etc.

Preheating the vapours of the ingoing pinene-free turpentine oil to 250° had a positive effect on the yields of p-cymene. Thus the heated vapours gave better yields, and a better control on the flow rate. Below this temperature for preheating the yields were low, while above this temperature, the yields of p-cymene remained constant.

The activity of the catalyst started decreasing after 20 times of its weight of the turpentine oil had passed through it. It could be regenerated by passing hot air or superheated steam at 400° for 2 hours. The catalyst could stand only three such regenerations. The catalyst could, however, be recovered and regenerated by treating it with dilute nitric acid and evaporating the solution to dryness.

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UTILIZATION OF D.D.T. WASTE FOR THE PREPARATION OF SCARLET GG FAST DYE SALT AND A NEW SULPHUR GREY DYE

MOHAMMAD ZAFAR SHAH, MOHAMMAD SARWAR, M.K. BHATTY AND KARIMULLAH

West Regional Laboratories, Pakistan Council of Scientific and Industrial Research, Lahore

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Introduction

A large quantity of p-dichlorobenzene is available in Pakistan as a bye-product of the D.D.T. manufacture. On the basis that 1,000 tons of D.D.T. are produced annually in West Pakistan and 1,400 tons in East Pakistan,[1] the quantity of the bye-product comes to about 75 tons/annum.[2] This quantity is further likely to increase with the setting up in West Pakistan of another D.D.T. plant of 1400 tons/annum[3] capacity.

This bye-product, which has so far been going waste, can be effectively utilized for the production of a number of such materials as are being imported at the expense of considerable foreign exchange. In an earlier communication,[4] it has been shown that p-dichlorobenzene can form the basis for the economic production of an amoebicide 5-chloro-7-iodo-8-hydroxyquinoline. Besides its use in the pharmaceutical field, p-dichlorobenzene can also become an important starting material for the preparation of a number of dye intermediates and dye salts.

Although several dyes such as, Congo Red, Direct Black, Direct Green, Direct Blue, Sulphur Black, Pak Direct Green, C Extra and Pak Direct Deep Black E Extra, are now being manufactured[5] within the country, a considerable quantity of these and many other dyes is still being imported. An idea of this import can be had from the fact that in 1962 alone, dyes worth Rs. 61.87 million were imported.[6] With this fact in view, attempts had, therefore, been made to use p-dichlorobenzene for the manufacture of dye stuff of both azo as well as sulphur type.

This paper accordingly deals with attempts which have been made for the utilization of p-dichlorobenzene in the production of an azo dye scarlet GG and a new sulphur dye. The former is a wellknown fast dye salt used in the azonic dyes

and pigments and in rapodigen colours.[7] It couples with a number of napthols to form dyes and pigments of different colours.[8,9] As far as it could be ascertained from literature, the latter dye has been prepared for the first time. The dye imparts a fast grey colour to cotton-fabrics and therefore, it promises to be of commercial importance.

Experimental

CHEMICALS

p-Dichlorobenzene:—The waste from the D.D.T. manufacture was subjected to distillation for the removal of a tarry matter. The distillate was chilled in an ice-bath and the resultant crystalline p-dichlorobenzene was filtered off from the residual o-dichlorobenzene.

Nitrating mixture: A mixture of nitric acid (1.41d) and sulphuric acid (1.84d) in the ratio of 42:58 respectively was employed for the nitration of p-dichlorobenzene.

Other chemicals are: Cast iron shavings, concentrated hydrochloric acid, sodium nitrite, zinc chloride, common salt, sodium hydroxide, sodium sulphide and sulphur.

All the chemicals used in these investigations were of commercial grade.

EQUIPMENT

Besides ordinary laboratory-ware the following equipment was employed: A stainless steel vessel of 2 litre capacity, fitted with reflux condenser and stirrer. An autoclave fitted with heating arrangement and a stirrer for the preparation of 4-chloro-2-nitrophenol from p-dichlorobenzene.[4]

Procedure for Scarlet GG

The nitration of p-dichlorobenzene into 2:5-dichloronitrobenzene was carried out according to the method described in the previous communication.[4]

Reduction of 2:5-dichloronitrobenzene into 2:5-dichloroaniline.—Thirty-five gram of 2:5-dichloronitrobenzene were heated with 50 g. of cast iron powder, 30 ml. of 2N hydrochloric acid and 200 ml. of water at 110° for 75 minutes in the iron reduction vessel fitted with a water condenser and a stirrer. The resulting product was steam-distilled to separate white crystalline 2:5-dichloroaniline (m.p. 50°, yield 92%).

Diazotization of 2:5-dichloroaniline.—A mixture of 16.2 g. of 2:5-dichloroaniline, 50 ml. concentrated hydrochloric acid and 200 ml. water was boiled in a 1-litre R.B. flask until a homogenous solution was obtained. The solution was cooled under tap water with vigorous shaking to obtain finely divided crystals of 2:5-dichloroaniline-hydrochloride. One hundred g. ice were added followed all atonce by 100 ml. of 1N sodium nitrite solution. Shaking of the mixture was continued until all of the precipitate had dissolved.

Formation of Fast Salt Scarlet GG.—While shaking the diazonium salt solution at 15-20°, 5 g. of zinc chloride were added in small portions. Sixty g. of common salt were added until the precipitation of the dye scarlet GG was complete. The precipitated dye was filtered off and dried at the room temperature. The filtrate was tested for the complete precipitation of the zinc chloride complex salt by taking a drop of the filtrate on a filter paper and adding another drop of a napthol solution near it. If there was any unreacted dizonium salt, a colour was produced at the junction of the two drops. In such case more zinc chloride was added to the filtrate. The yield of the zinc complex was 98%. The overall yield of scarlet GG starting from p-dichlorobenzene was 80 percent.

Procedure for Sulphur Grey Dye

Treatment of 4-chloro-2-Nitrophenol with Sodium Sulphide and Sulphur.—In a 2-litre R.B. glass flask, 30. g. of 4-chloro-2-nitrophenol as prepared from p-dichlorobenzene,[4] 15 g. of sodium hydroxide and 150 ml. of water were added and heated at 80° for 30 minutes. The solution was cooled to 45° and mixed with a solution of 72 g. of sulphur powder in 160 ml. of water containing 160 g. of crystalline sodium sulphide. The temperature was raised to 60°C. and the volume brought up to 600 ml. The temperature was immediately raised to 80°, and then to 150° in the course of 2 hours. The mixture was refluxed for 32 hours and thereafter diluted with 300 ml. of water. Air was passed into the reaction mixture at 60-70° until a grey coloured precipitate was obtained. The precipitate was filtered off and dried at 70°. Weight of the precipitated dye was 210 g. (Yield 80.2%).

Discussion

Although p-dichlorobenzene is known as an important starting material for the manufacture of a number of dye intermediates and dye salts,

yet the details of the methods are always obscure and covered by patents. The methods are a trade secret and thus in the preparation of scarlet GG from p-dichlorobenzene, all the technical know-how has been worked out independently in these Laboratories. On the basis of estimates carried out here, each pound of scarlet GG will cost Rs. 2.00. The market price of the imported dye is Rs. 3.00 per pound. Therefore, the manufacture of scarlet GG within the country can be undertaken economically.

The sulphur grey dye was prepared from the p-dichlorobenzene for the first time. The well-known dye sulphur black is obtained as a result of the reaction of sodium hydroxide, sulphur and sodium sulphide on 2:4-dinitrochlorobenzene.[10] The new sulphur grey dye has, however, been obtained by using 4-chloro-2-nitrophenol instead of 2:4-dinitrochlorobenzene. As the starting material in both the cases is different, the dye obtained, therefore, imparts different shades. The new dye was found most suitable for cotton fabrics. It is fast to light and soap washing. Per pound cost of the dye was estimated at Rs. 1.50 which is much lower than any of the sulphur dyes available in the market. Sulphur black is sold in the market at a price of Rs. 2.50 per pound.

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PRELIMINARY SURVEY OF THE MEDICINAL PLANTS OF BALUCHISTAN

S. M. A. KAZMI

North Regional Laboratories, Pakistan Council of Scientific and Industrial Research, Peshawar

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Although Baluchistan is quite rich in vegetation, so far very little is known about its botanical wealth. It presents absolutely a distinct picture of the flora as against the rest of West Pakistan. The area represents the Flora of Iranian Highlands which, in the west is, spread right up to the Southern districts of the Soviet Union and in the south-west up to Iraq. It was only in the late 19th Century that three botanists namely Stocks, Lace and Burkill could explore the area and make plant collections. A negligible part of these incomplete collections have been preserved and is available for reference at the herbariums of Kew, Vienna and Geneva.

In this connection, we in Pakistan, have practically no authentic material or literature except a publication "Working List of the Plants of Baluchistan" by Burkill, which too is out of print now. Thus, very little is known of the economically important plants found in Baluchistan.

Baluchistan is developing side-by-side with the rest of other units of West Pakistan. It, nevertheless, deserves special attention in the matter of exploitation of its natural resources, including medicinal plants.

In view of the foregoing circumstances, and in order to derive full advantage of the visit to Pakistan of a team of experienced botanists consisting of a staff member of the Royal Botanic Gardens, Edinburgh, and the Director of the Natural History Museum Vienna, which intended to botanically explore Baluchistan, the Director, North Regional Laboratories of the Pakistan Council of Scientific and Research, allowed the present author to join this party during the months of April and May 1965 for the collection of plants with particular reference to those of economic value.

Plant collections have been made between Bela and Hussheb, south of Turbat, Pasni, Gwadar and the surrounding areas for the first time.

Specimens have also been collected from the tribal area between Fort Sandamen and Dera Ismail Khan, for the first time.

The entire collection has been catagorised into three parallel sets. One set is meant to be preserved at the Herbarium of the Royal Botanic Gardens, Edinburgh; one at the Herbarium of the Natural History Museum of Vienna and the other at the Herbarium of the North Regional Laboratories, Pakistan Council of Scientific and Industrial Research, Peshawar.

Work on the mounting and identification of these plants is in progress and it is expected that a series of new medicinal plants, from the areas mentioned above, will become known in due course.

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GERMINATION OF UREDOSPORES OF PUCCINIA GRAMINIS PERS. ON CELLOPHANE PAPER

SHAKIL AHMAD KHAN AND M.M.S. MALIK

Agriculture Research Institute, Tandojam

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Uredospores of *Puccinia graminis* germinate readily in water or moist air, and various methods are being used for testing their germination. The common method of germinating the spores in a drop of water is, no doubt, simple but the present authors have found that comparatively better germination is obtained if the spores are floated on a piece of moist cellophane paper in a Petridish.

Small pieces of cellophane, 1 or 2 mm. square, are cut and floated on water in a Petridish. Uredospores are dusted on these pieces, and the lid of the Petridish is replaced. For examination, the cellophane pieces are picked up by means of a forceps, and examined on a glass slide in a drop of water or lacto-phenol.

In a germination test conducted in the laboratory (Temp. 63—71°F.) with the freshly collected uredospores of *Puccinia graminis tritici* Eriks. and Henn. from field (Temp. 13.3°C.) the spores on cellophane gave 93 percent germination in three hours. Germination starts within an hour, and a germ-tube length of 27μ is reached in two hours.

A STUDY OF FUNGUS FLORA OF KARACHI CANTT. SOIL

S. R. H. RIZVI

Central Laboratories, Pakistan Council of Scientific and Industrial Research, Karachi

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Introduction

The study of soil micro-organism has been of great interest in many countries, but in our country, very few workers have undertaken the study of this branch of Mycology. In Indo-Pakistan sub-continent the first study of soil fungi was made by Butler.[1] Later Thakur and Norris[2] described fungi from Madras soil and reported 22 species. Mason[3] from the paddy field in Burma isolated 4 species of *Aspergillus* and one species of *Acrothecium*, Chaudhary and Sachar[4] described 32 species from Panjab soil, Galloway[5] has given generic description of soil fungi isolated from Indian soil, Roy[6] has described soil fungi from paddy field of Bengal; Sadasivan and Subramanian[7] described their study of soil borne Fusaria, Saksena[8–11] recorded two new genera and two new species from Indian soil, Ahmad[12] published the list of fungi recorded from West Pakistan which included the fungi isolated from soil.

Experimental

Soil Sampling.—A plot of virgin land was selected at the Central Laboratories, Pakistan Council of Scientific and Industrial Research, Karachi Cantt., Karachi. Soil samples were taken from 3 to 6 inch depth from the surface. For collection of soil samples, a small profile was dug and sterilized specimen tubes ($4'' \times \frac{1}{2}''$) were pushed horizontally into a prepared face.

Method of Isolation.—Two methods with Czapek Dox agar and Jensen medium were followed in the process of isolation of fungi from soil samples.

(a) Direct Inoculation Method: About 0.5 g. of soil was added to about 20.0 ml. cold medium in a sterilized Petri plate and soil particles were dispersed throughout the plate by slow shaking and constant rotating, before the medium solidified.

(b) Dilution Plate Method: About 1.0 g. soil was transferred to 20.0 ml. of sterilized water in a 100.0 ml. conical flask, the flask was shaken to

make a uniform solution. 1.0 ml. of this solution was transferred to a sterilized Petri plate and 20.0 ml. of cold medium was added. The solution was dispersed throughout the plate by slow shaking and constant rotating as before.

The plates were incubated at 28°C. After the colonies appeared, isolation of different forms were made on Czapek and Jensen agar slants. Identifications were made on Potato dextrose, Czapek dox, and Jensen medium with the help of Thom and Raper,[13] Gilman,[14] Raper and Thom,[15] Barnett,[16] Bessey,[17] and Smith.[18] Confirmation of different species were obtained from Commonwealth Mycological Institute, Kew, Surrey, England.

Observations

Fungi Isolated.—The list of fungi isolated from the soil have been presented below. These include 78 species with 10 nontypical strains belonging to 32 genera. These isolate also include 24 fungal records for this region and three quite new species.

List of Fungi Isolated

PHYCOMYCETES

Oomycetes: (1). *Pythium sp.*

Zygomycetes: (2). *Absidia sp.*,(3). *Actinomucor sp.*, (4).*Choanephora cucurbitarum.*, (5).*Circinella mucorides.*, (6). *Cunninghamella echinata.*, (7). *Cunninghamella elegans.*, (8). *Mucor circinelloides.*, (9). *Mucor globosus.*, (10). *Rhizopus arrhizus.*, (11). *Rhizopus nigricans.*

ASCOMYCETES

Pyrenomycetes: (12). *Chaetomium sp.*

FUNGI IMPERFECTI

(13).*Alternaria stemphyliodes.*,(14). *Alternaria tenuis.*, (15).*Alternaria humicola.*,(16). *Aspergillus amstelodami.*, (17). *Aspergillus awamori.*, (18). *Aspergillus candidus.*, (19). *Aspergillus chevalieri.*, (20). *Aspergillus flavipes.*, (21a). *Aspergillus flavus.*, (21b). *Aspergillus flavus*—Sclerotial strain., (21c). *Aspergillus flavus*—smooth conidiophores and smooth spores., (22). *Aspergillus fumigatus.*, (23) *Aspergillus luchuensis.*, (24a) *Aspergillus nidulans.*,(24b). *Aspergillus nidulans*-conidial st te only., (25). *Aspergillus niger.*, (26). *Aspergillus ochraceus.*,(27). *Aspergillus restrictus.*, (28). *Aspergillus ruber.*, (29). *Aspergillus sulphureus.*, (30). *Aspergillus sydowi.*, (31). *Aspergillus tamarii.*, (32a). *Aspergillus terreus.*, (32b). *Aspergillus terreus*—Floccose and sparsely sporing strain., (33). *Aspergillus ustus.*, (34). *Aspergillus versicolor.*, (35). *Aspergillus violaceo-fuscus.*, (36). *Aspergillus wentii.*, (37). *Cephalosporium sp.*, (38). *Cladosporium cladosporiodes.*, (39). *Cladosporium spaerospermum*—without bright yellow pigments., (40). *Cladosporium sp.* novo., (41). *Corticium solani*—somewhat a typical., (42). *Curvularia lunata.*, (43). *Curvularia pallescens.*, (44). *Curvularia spicifera.*, (45). *Cylindrocarpon hetronemum.*, (46). *Cylindrocarpon radicola.*, (47). *Dactylaria purpurella.*, (48). *Epicocum nigrum.*, (49). *Fusarium dimerum.*, (50). *Fusarium equiseti.*, (51). *Fusarium oxysporum.*, (52). *Fusarium sambucinum.*,(53). *Fusarium semitectum.*, (54).*Fusarium solani.*, (55). *Fusarium sporotrichiodes.*, (56). *Helminthosporium halodes* var. *tritici.*, (57). *Helminthosporium hawaiiense.*,(58). *Humicola fusco-arta.*,(59).*Myrothecium verrucaria.*, (60). *Paecilomyces fusisporus.*, (61). *Penicillium citrinum.*, (62). *Penicillium expansum*—not absolutely typical., (63). *Penicillium janthenellum.*, (64).*Penicillium lilacinum.*, (65). *Penicillium oxalicum.*, (66).*Penicillium ruberum.*, (67).*Penicillium spinulosum.*, (68).*Penicillium sublateritium.*, (69). *Penicillium vinaeceum.*, (70). *Pestalotiopsis sp.*,(71). *Phoma sp.* novo., (72). *Rhinocladiella sp.*, (73).*Scopulariopsis* sp., (74). *Stachybotrys arta.*, (75). *Stysanus sp.*—Without coremia., (76). *Trichoderma koningi.*,(77). *Trichoderma virides.*, (78). *Trichurus spiralis.*,

The Phycomycetes in this isolation are represented by eleven species belonging to eight genera, one species belong to Oomycetes and the remaining ten species to seven genera belonging to Zygomycetes. *Circinella mucoriodes* belonging to Zygomycetes is the only new record for this region.

Ascomycetes is represented by only one species (*Chaetomium sp.*)., which is a non-typical strain belonging to Pyrenomycetes. Fungi belonging to Basidiomycetes were not recorded in any of the isolation, and hence we lack a major group of fungi.

The remaining 66 species with 9 non-typical strains, 23 new records for this region and three quite new species belonging to 23 genera are the members of a varied group of fungi known as Fungi Imperfecti. New records for this region include numbers 13, 16, 17, 27, 28, 36, 38, 45, 46, 52, 55, 56, 58, 59, 64, 66, 67, 68, 69, 70, 72, 74 and 78 as enumerated in the List of Fungi. Non-typical strains consist of numbers 12, 21b, 21c, 24b, 32b, 39, 41, 62, 70 and 78 of the List.

All the isolates belonging to Phycomycetes are typical with normal morphology and growth. No. 12 (List of Fungi) the only isolate belonging to Ascomycetes is a nontypical strain as ascocarps

in this strain were quite sterile and no spores were found in any of their ascocarps.

The remaining 9 non-typical strains belonging to Fungi Imperfecti have been briefly described below with their significant remarks.

(21b). *Aspergillus flavus*—Sclerotial strain.

(21c). *Aspergillus flavus*—Smooth conidiophore and smooth spores otherwise typical of species.

(24b). *Aspergillus nidulans*—Conidial state only.

(32b). *Aspergillus terreus*—Floccose and sparcely sporing strain.

(39). *Cladosporium sphaerospermum*—Morphologically identical with the species, but without bright yellow pigments

(41). *Corticium solani*—Somewhat atypical strain.

(62). *Penicillium expansum*—Not absolutely typical strain.

(70). *Pestalotiopsis sp.*—The spores borne in true pycnidia which occasionally become divided and similar to *Phomopsis* fructification in form. In addition the morphology of conidia is quite unlike any *Pestalotiopsis* isolated from soil

(78). *Stysanus sp.*—The pattern of conidial formation and shape of conidia are strongly reminiscent of the genus, but no cornia have been formed in this culture.

Three isolates number 40, 71 and 73 in the List have been considered as new species as per comments from the Commonwealth Mycological Institute, Kew, Surrey, England.

(40). *Cladosporium sp.*—This does not match any of the species we have in culture, but very little work has been done on tropical species of *Cladosporium* and this may well be a species known so far only on natural substrata.

(71). *Phoma sp.* "An unusual species not represented in this herbarium which is distinguished by its indistinct ground mycelium and rose pink spores masses."

(73). *Scopulariopsis sp.* "Does not match with any species known to us."

The detailed studies regarding these three new fungi have been dealt with in a seperate paper.

Detailed study of the climatic conditions of this region had earlier been made by Pithawala.[19] This report of 78 species belonging to 32 genera shows that fungi are cosmopolitan and further studies may add many more already reported from other regions. Isolation of 10 non-typical strains and three quite new species are addition to fungal record. Isolations of 21 species and 4 non-typical strains of *Aspergillus* and 9 species of *Penicillium* during this study strongly support the previous work (Waksman *et al.*[20]) that for warmer climate Aspergillus is more dominant over *Penicillium*. *Fusarium*, which occupies the third place in this study is represented by 7 species, the remaining 37 species belonging to 29 genera.

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