SHORT COMMUNICATIONS

INVESTIGATIONS ON FARIDPUR PEAT

Part II.—Fraction and Infra-Red Studies of Peat Bitumen

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(Received November 16, 1964)

In the previous communication[1] were described various products from peat that were isolated and tested. It is felt that Faridpur peat being the only major indigenous solid fuel in East Pakistan, requires more study. The study of the chemistry of peat as a whole and its constituents and structure has long been made by many workers.[2] However, the chemistry of peat bitumen has not received much attention. Various workers isolated bitumen, extracting powdered coal by ethanol-benzene mixture in varied ratios. The yield of bitumen varied between 3-19 percent; it melted at 70-100°C. and contained carbon between 70-80 percent and hydrogen 8-14 percent. These figures correspond to the Faridpur peat bitumen fairly well.

The Faridpur peat bitumen was first fractionated by methanol as methanol soluble and methanol insoluble parts; the former portion was further fractionated by chromatography with an anion exchanger and eluted by methanol, methanol-acetic acid and acetic acid with the expectation that it would separate various groups of compounds. The infra-red spectra were recorded from 4000-650 cm^{-1} frequency. The peaks were not very conclusive towards low frequency zone which, the authors find, has been a characteristic of peat and coal spectra.

Experimental

Extraction of Bitumen: About a ton of peat from Faridpur was supplied by the East Pakistan Industrial Development Corporation. One pound of it was air dried to a moisture content of 12.4 percent and powdered to pass through a sieve of 60 mesh/cm^2. 260 g. of the powder was extracted by Soxhlet extraction with 300 ml. of benzene-methanol (60:40). The yield of bitumen was 19.76 g. i.e. 8.67 percent of the moisture free peat. It melted at 78°C. and on analysis it showed C, 77.3 and H, 8.5 percent.

Fractionation of the Bitumen.—10.1 g. of bitumen was treated with 150 ml. of methanol for Soxhlet extraction. After extraction, the methanol insoluble part was found to be 5.7 g. (56%). The methanol extract 4.4 g. (44%) was cooled to room temperature and filtered. The residue yielded waxy materials 0.24 g. (2.4%). The filtrate was fractionted by chromatography on a column (10 cm. long, 1 cm. diameter) of the ion exchanger II (slightly alkaline anion exchanger, E. Mercks No. 4766). Elution was effected with methanol followed by 25 percent glacial acetic acid in methanol and then with glacial acetic acid until no more elution with a particular eluant was possible. The fractions thus chromatographed were dried and weighed. About 2.64 g. (60%) of the material charomatographed was methonol eluted; 0.22 g. (5%) came with methanol-acetic acid and about 0.05 g. (1%) with acetic acid. The remainder could not be eluted.

Infra-red spectra were recorded by a Beckman IR-4 spectro-photometer. Spectra of the following samples were taken: peat, parent bitumen, methanol insoluble of bitumen, precipitate on cooling the methanol extract of bitumen and fractions eluted from the anion exchange column by methanol and methanol-acetic acid. For peat, potassium bromide pellet carbon tetrachloride suspension and for others, spectra grade chloroform were used. The frequencies of the spectra are shown in the following:

Frequencies of Infra-Red Spectra of Peat and Bitumen Fractions

1. Peat, max^{KBr} in cm^{-1} :3448-3226 (wide), 2941(m), 2865(m), 1666-1538(wide), 1408-1351 (wide);

2. Parent bitumen, $^{v}max^{CHCl_3}$ in cm^{-1}: **2932(s)**, 2874(s), 1712(m), 1499(w), 1459(m) **1418(w)**, 1364(w), 1175-1125 (wide), 917(w);

3. Methanol-insoluble fraction of peat bitumen, $^{v}max^{CHCl_3}$ in cm^{-1}: 3425(w), 2932(s), **2868(s)**, 1712(m), 1597(w), 1459(m), 1412(m), 1177(w);

4. Precipitate from the methanol soluble extract of bitumen, $\nu_{max}^{CHCl_3}$ in cm^{-1} : 2932(s), 2876(s), 1704(s), 1608(w), 1459(m), 1416(w), 1163(w);

5. Methanol soluble fraction after chromatography by slightly alkaline anion exchanger, E. Mercks No. 4766: (a)methanol eluted portion, $\nu_{max}^{CHCl_3}$ in cm^{-1}: 3448(m), 2985(s), 1751(s), 1479(m), 1393(m); (b) acetic acid-methanol eluted portion, $\nu_{max}^{CHCl_3}$ in cm^{-1} :3448(m), 2985(s), 1754(s), 1483(m).

Results and Discussion

The percent bitumen in the Faridpur peat has been found to be 8.67 on moisture free basis; Ordinarily, bitumen content[3] in the peat varies between 3-19 percent, so Faridpur peat has undergone average humification. The frequencies in the infra-red spectra of peat and its bitumen fractions have been characterised in Table 1.

TABLE 1.—CHARACTERISATION OF THE FREQUENCIES OF FEAT AND ITS BITUMEN FRACTION IN THE INFRA-RED SPECTRA.

Frequency, in cm^{-1}	Characterisation
~3448	OH Group
2985, 2967 and 2876	CH Stretching frequencies of CH_3
2932 and 2868	CH Stretching frequencies of CH_2
1754 and 1751	CO Stretching aliphatic ester
1712 and 1704	CO Stretching aromatic ester
~1600 ~1500	Aromatic ring frequency
1483, 1479, 1475 and 1459	CH Deformation frequencies of C-CH_3, -CH_2-
~1391, 1393 and 1400-1350	CH Deformation frequencies of C-CH_3, C-$(CH_3)_2$ and -$C(CH_3)_3$
1175-1225	Alkyl ethers -CH_2O-CH_2

The presence of aliphatic structure is shown by the peaks at 2985, 2967, 2876, 2932 and 2868 cm^{-1}. Peaks at 1483, 1479, 1475 and 1459 cm^{-1} are further proofs of aliphatic types. Aromatic structure is shown by bands at 1600 and 1500 cm^{-1} although no aromatic C-H stretching was indicated by the absence of peak at 3030 cm^{-1}. Presence of oxygen is indicated by the peak around 3448 cm^{-1} for hydroxyl group, at 1754, 1751, 1712 and 1704 cm^{-1} for carbonyl groups and at 1175-1125 cm^{-1} for alkyl ether. In general, peaks towards lower frequency are less distinct. Spectra show that in bitumen, constituents of aliphatic nature containing carbonyl groups are more concentrated than they are in the peat.

The spectra of the methanol-insoluble fraction and the precipitate from the methanol soluble extract of the bitumen are quite similar. The peaks are sharp and distinct. Both of them contain aromatic constituents and their esters, in contrast to the methanol soluble fraction.

The spectra of the bitumen and the methanol soluble fraction are also similar; the peaks are sharper in the latter containing more of the alipaphatic constituents with carbonyls.

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STUDIES ON FAGONIA CRETICA LINN

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(Received January 25, 1965)

Fagonia cretica Linn[1] (N.O. Zygophyllaceae) is a small spiny under-shrub with stiff branches, often more or less prostrate. It has tiny rose-coloured solitary flowers found almost all the year

round. It is a very common plant, widely distributed throughout West Pakistan. The aqueous decoction of leaves and young twigs is a popular remedy for the treatment of skin lesions (boils and abscesses), particularly amongst children. It is described as astringent, febrifuge and a cure for any disorders arising from poisoning. Of late, the plant has assumed greater importance and is claimed to be a remedy for tumours and cancer in its early stages. Preliminary pharmacological tests[2] have, however, shown that the freeze-dried powder obtained from a 60 percent ethanol-water extract of macerated drug is not effective against experimental tumours. It was tried and found to have no activity against S. 180 and Ehrlich ascites tumours, or against AKR luekaemia in mice at a dose of 500 mg./kg. per day, subcutaneously or intraperitoneally for five days. The drug is believed to be palliative rather than curative and any benefit experienced is likely to be due to the surface healing of the sore spot.

As no previous chemical investigation of the plant has been reported, it was considered desirable to undertake a detailed examination of the plant. The material was collected from Peshawar District and used fresh. As a result of this work, the authors have been able to isolate a glycosidal bitter principle as a light cream-coloured powder, freely soluble in water and ethyl alcohol. This complex, on hydrolysis with alcoholic hydrochloric acid, gave a crystalline product, m.p. 305-307°, almost insoluble in all organic solvents and only difficultly soluble in boiling methyl and ethyl alcohols from which it crystallises out on slow evaporation or distillation of the solvent. Work on the chemistry of this bitter complex is in progress and will be reported in due course.

The present paper deals with the petroleum ether-soluble fraction of the alcoholic extract of the plant, from which two crystalline fractions have been obtained: (A) m.p. 75-76°. (yield 0.007%) and (B) m.p. 140-41°. (yield 0.012%). On the basis of combustion analysis and molecular weight determination the first fraction is provisionally formulated as $C_{27}H_{56}O$. It is non-steroidal and its acetyl derivative melts at 62-63°. The second fraction, m.p. 140-41°, is provisionally formulated as $C_{27}H_{46}O$. Its acetyl derivative melts at 117-120°. It is steroidal in character. Both the fractions did not decolorise potassium permanganate or bromine water indicating the absence of olefinic double bond. Owing to lack of adequate material, the acetyl derivatives of both the fractions could not be analysed.

Experimental

The fresh plant (dry weight, 4 kg.) after collection, was finely chopped and soaked in ethyl alcohol (90-95%). The extracts were drawn after every 24-hour. After three extractions, the plant material had practically lost its bitter taste. The combined extracts were concentrated in a cyclone evaporator and the last traces of alcohol and moisture removed under reduced pressure. The temperature at no stage was allowed to rise beyond 55-60°. The dark-green semi-solid mass was repeatedly extracted with acetone. The dark-brown residue insoluble in acetone was worked for the isolation of the bitter complex, to be described in a subsequent communication. The acetone-soluble fraction after complete removal of the solvent was extracted with petroleum ether (40-60°). The petroleum ether soluble fraction was treated with activated charcoal, dried over anhydrous sodium sulphate and distilled.

The dark-green liquid was adsorbed on a column (24 cm. × 4 cm) containing activated alumina (200 g. May and Baker) and eluted with petroleum ether. No separation was affected except for retaining the chlorophyll. All fractions were, therefore, combined. The sticky residue obtained on removal of the solvent was dissolved in acetone which, on standing for some time, deposited a white crystalline residue and was decanted off. The residue was repeatedly crystallised from a mixture of methyl alcohol and acetone (10:1) and finally gave colourless silky crystals, m.p. 75-76°. Found: C, 82.01; H, 13.42. $C_{27}H_{56}O$ requires: C, 81.74; H, 14.23. molecular weight, 385 (Rast method).

It is easily soluble in most of the organic solvents and fairly soluble in hot ethyl and methyl alcohol. It did not decolourise a solution of potassium permanganate in acetone or absorbed bromine, indicating the absence of unsaturation.

Its infra-red spectra [3] (Fig. 1) shows the peaks at (719, 730) (four adjacent straight chain methylene group), 1060, 1460 (-CH_3 and-CH_2), 2830, 2900 (-CH_2) and 3300 (OH) cm-^{1}.

The compound (A) was acetylated in the usual manner and was crystallised from methyl alcohol. The crystals obtained in colourless plates m.p. 62-63°.

The combined mother-liquors were concentrated and taken in a mixture of alcohol and acetone (10:1). The crop of crystals obtained melted at 140-41°C. Found: C, 83.99; H, 11.91.

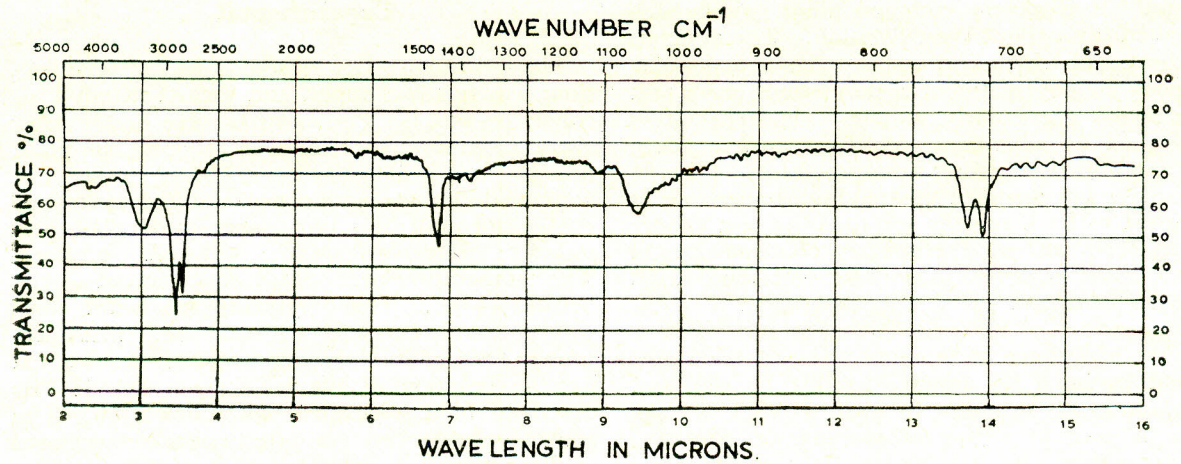

Fig. 1.—Showing Infra-red Spectra of Compound A.

C_{27} $H_{46}O$ requires: C, 83.87; H, 11.91. Molecular weight, 385 (Rast method).

It is easily soluble in ether in the cold but dissolves in other organic solvents when heated. It does not take up bromine nor does it decolourise potassium permanganate, indicating the absence of unsaturation. It, however, gave a positive Liebermann-Burchard reaction indicating its steroidal nature.

The infra-red spectra (Fig. 2) shows peaks at 796, 840, 958, 970, 1021, 1052, 1380 (C-CH_3 Symmetrical), 1460 (-CH_2 and -CH_3), 2910 (-CH_2) and 3400 (-OH) cm^{-1}.

Acetyl derivative of (B) was prepared by the usual method. The sticky residue was crystallised from methyl alcohol giving colourless crystals m.p. 117-20°C.

Summary

A glycosidal bitter complex has been isolated from the fresh drug, which, on hydrolysis gave a crystalline product m.p. 305-307°C. Besides, two

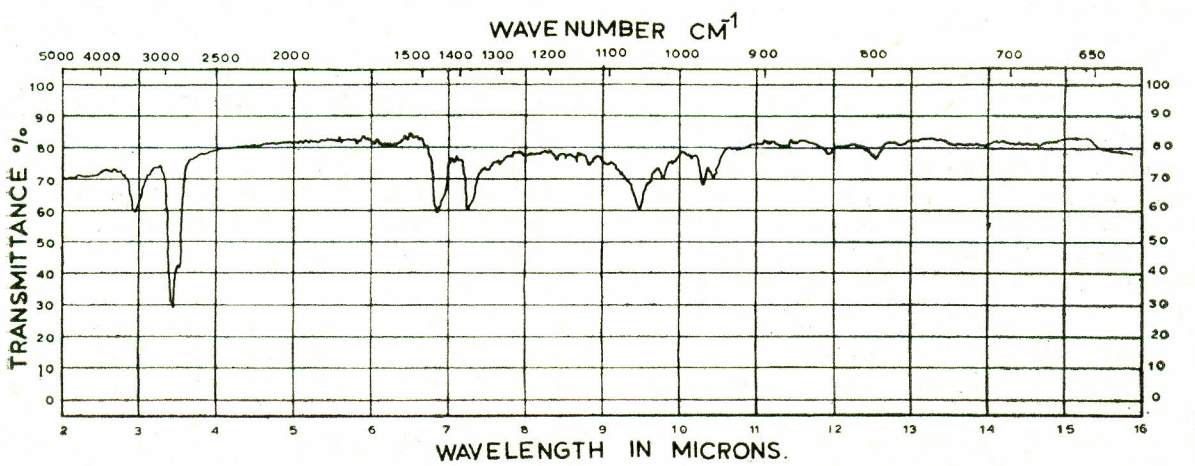

Fig. 2.—Showing Infra-red Spectra of Compound B.

petroleum ether-soluble fractions have been obtained, one melting at 75-76°C. is fomulated as $C_{27}H_{56}O$ and the second melting at 140-41°. is formulated as $C_{27}H_{46}O$ on the basis of their combustion analysis and molecular weight determination. The first fraction is aliphatic, while the second fraction is steroidal in character.

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SELECTIVE OXIDATION OF STEROIDAL ALCOHOL BY DIMETHYL SULPHOXIDE

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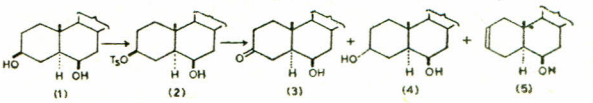
(Received April 8, 1965)

Dimethyl sulphoxide oxidation of various steroidal sulphonic esters has shown that ketones were obtained in yield of preparative significance only with sulphonic esters of 5α -cholestan-3β -and 7β -ols.[1] Since double bonds remain unaffected, this procedure may be useful for selective oxidation of 3β -and 7β -steroidal alcohols. Alcohols also remain unaffected by dimethyl sulphoxide, much more drastic condition being required to dehydrate even tertiary aliphatic alcohols.[2] As hydroxyl groups in different parts of the steroid skeleton can be selectively tosylated (for example 6β -and 7α -hydroxycholestane do not form toluence-p-sulphonates while 3β -and 7β -hydroxy cholestanes can easily be converted to their tosylates) it is feasible to oxidise them selectively.

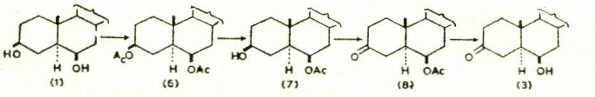
For selective solvolytic oxidation of steroidal alcohols the author chose 3β ,6β -dihydroxy-5α -cholestane(1). Treatment of 3β ,6β -dihydroxy-5α -cholestane with toluene-p-sulphonyl chloride at room temperature furnished 3β -tosyloxy-6β -hydroxy-5α -cholestane in 61 percent yield after crystallisation.[3] The 3β -tosyloxy-6β -hydroxy-cholestane(2) was treated with dimethyl sulphoxide under the usual reaction conditions.[1] The crude



(1) (2) (3) (4) (5)

product on crystallisation gave 6β-hydroxy-5α-cholestan-3-one(3) in 30 percent yield and the mother liquor afforded more 6β -hydroxy-5α-cholestane-3 -one(3) (15 percent) after chromatographic separation. A total yield of 70 percent of the ketone(3) was thus obtained as compared to 64 percent yield of 5α -cholestan-3-one after solvolytic oxidation of 3β -tosyloxy-5α -cholestane.[1]

The conventional method of obtaining 6β -hydroxy-5α -cholestan-3-one(3) from 5α -cholestan-3β, 6β -diol usually involves four steps.[4,5] The acetylation of the dihydroxycholestane (1) gave 70 percent yield of diacetate(6).[2] Partial hydrolysis of 3β, 6β -diacetoxycholestane was found to give 70 percent yield of 3β -hydroxy 6β -acetoxy-5α -cholestane(7) after chromatographic separation. The oxidation of monoacetate(7), usually affords 95 percent yield of 6β -acetoxy-5α -cholestan-3-one(8),[3] which on hydrolysis gives 6β -hydroxy-5α -cholestan-3-one(3). The overall yield



(1) (6) (7) (8) (3)

of ketone(3) by this method is not more than 40 percent. The author's procedure therefore involves only two steps and the overall yield of 6β -hydroxy-5α -cholestan-3-one(3) (42 percent) is quite reasonable.

Experimental

Reaction between 3β, 6β -dihydroxy-5α -cholestan-3β -toluene-p-sulphonate and dimethyl sulphoxide:—The dihydroxy monoester (1.203 g.) was heated in dimethyl sulphoxide (10 ml.) in the presence of collidine (0.38 g.), at 98-100°, for three hours. After working up the product in ether in the usual manner, a solid (851 mg.) was obtained, which on crystallisation from acetone, furnished 6β -hydroxy-5α -cholestan-3-one (261mg.) (30 percent), m.p. 187-190°. It was homogeneous

on a chromatoplate. The mother liquor (590 mg.) was chromatographed on alumina (19 g.). Elution with light petroleum/benzene (1:1 and 1:4) and benzene gave a mixture of four compounds (112 mg.). Elution with benzene/ether (4:1 and 1:1) gave a solid (438 mg.) which on crystallisation from acetone furnished 6β -hydroxy-5α -cholestan-3-one (278 mg., m.p. 186-190°). The mother liquor and the latter fractions from the alumina column after elution with ether were rechromatographed on two silica gel plates. Three layers were separated. The fast moving layer gave 2 mg. of syrup, the middle layer gave 6β-hydroxy-5α -cholestan-3-one (69.3 mg.) (8 percent) and the bottom layer gave a syrup (50 mg.) which was mostly 3α, 6β -dihydroxy-5α -cholestane according to t. l.c. The total yield of 6β-hydroxy-5α -cholestan-6-one was therefore 70 percent.

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THE REACTION BETWEEN 3α, 5α-CYCLOCHOLESTAN-6β-CHLORIDE AND DIMETHYL SULPHOXIDE*

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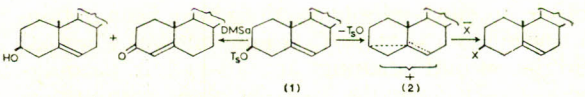
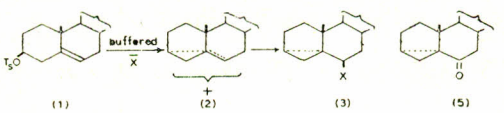
(Received April 8, 1965)

Dimethyl sulphoxide has been known to oxidise cholest-5-ene-3β-tosylate to cholest 4-ene-3-one in the presence of base.[1,2] Usually nucleophilic reactions involving cholesteryl tosylate(1) nearly always proceed by unimolecular heterolysis of the tosylate group to give a mesomeric cation(2), which in turn is attacked by

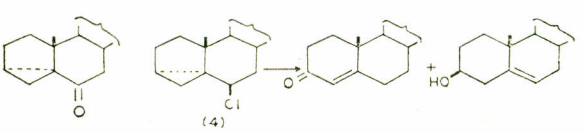


the nucleophiles to give products with retention of configuration.[3] In buffered media the nucleophile may attack the mesomeric cation at the 6 position to give rise to 6β -substituted 3α, 5α -*cyclo*cholestane(3).[4] But none of the 3α, 5α cyclo cholestan -6-one(5) was formed, when the tosylate(1) was treated with dimethyl sulphoxide in the presence of collidine.[1]

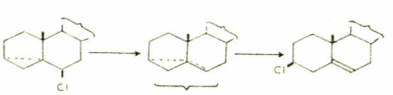


In order to study the possibility that the reaction did involve initial heterolysis of the tosylate group to give a mesomeric cation(2) the author investigated the reaction of 6β -chloro-3α, 5α -*cyclo*cholestane(4) with dimethyl sulphoxide under the usual solvolytic condition.[1] This compound is known to ionise with extreme ease to give the mesomeric ion(2).[4]

Reaction of 6β -chloro-3α, 5α -*cyclo*cholestane with dimethyl sulphoxide in the presence of collidine gave 50 percent of cholest-4-ene-3 one (determined spectrometrically). Thin layer chromatography showed the presence of hydrocarbon (20 percent), some cholesterol (5 percent), and 3α, 5α -cyclochol estan-6 β-ol in the reaction product, and absence of 3α ,5α -cyclocholestan-6-one(5). Therefore these results showed that 6β -chloro-3α 5α *cyclo*cholestane



rearranged to cholesteryl 3β -chloride *via* a mesomeric ion (2) and gave the same type of products



as were obtained in the case of cholesteryl 3β-tosylate(1).

Experimental

All solvents were dried before use. Merck's silica gel G was used for thin layer chromatography

*This paper is based on the work carried out by the author in the Chemistry Deparment, Sheffield University, U.K.

on glass (chromatoplate technique). Thin layer chromatography on a preparative scale was performed on glass plates 25 cm. square with layer of absorbent 1 mm thick (loading 100-120 mg. of substance per plate). Optical rotations were determined in chloroform on an automatic polarimeter. Ultraviolet spectra were taken on Perkin Elmer spectrometer.

3α, 5α -Cyclocholestan-6β -lo. -A mixture of cholesteryl tosylate (19.71 g.), potassium acetate (15.67 g.), acetone (300 ml.) and water (75 ml.) was refluxed for 12 hours, poured into water, extracted with ether, washed, dried (Na_2SO_4) and evaporated to give a residue (14.12 g.) which was chromatographed on alumina (420 g.). Elution with pentane gave hydrocarbon (167 mg.), whilst elution with pentane/benzene (1:1) gave pure 3α, 5α -*cyclo*cholestan-6β-ol (6.35 g.) m.p. 66-68°. Elution with benzene gave more 3α, 5α -*cyclo*cholestan-6β -ol (3.57 g.), m.p. 66-70° (Lit. m.p. 66-67°). Elution with ether/benzene (1:1) gave a mixture of cholesterol and 3α, 5α -*cyclo*cholestan-6β -ol (1.73 g.). Further elution gave pure cholesterol.

6β -Chloro-3α, 5α -cyclocholestane.—To a solution of 3α, 5α -cyclocholestan-6β -ol (4.32 g.) in dry ether (40 ml.) at 0° was rapidly added thionyl chloride (0.87 ml.). After two minutes at 0° the ether was evaporated under reduced pressure at room temperature. The white residue was dissolved in 40 ml. of dry petroleum ether (b.p. 40-60°) and rapidly filtered through calcium carbonate under suction, The petroleum solution was diluted with dry acetone and most of the petroleum ether distilled off under reduced pressure at room temperature. It was cooled to -75° when a white crystalline substance was deposited on the flask. This was collected, and dried over phosphorus pentoxide under vacuum (22 mm.). It melted at 73-81°, $[\alpha]_D$ +26.4. The specific rotation of the purest 6β -chloro-3α , 5α -cyclocholestane (86.6%) recorded by Winstein and Kosowar was +27.5°, and cholesteryl chloride has $[\alpha]_D^{31}$ Therefore the author's sample was at least 79 percent pure 6β -chloro-3α, 5α -cyclocholestane.

Reaction between 6β -chloro-3α, 5α -*cyclo*cholestane and dimethyl sulphoxide.- -6β-Chloro-3α, 5α -cyclocholestane (327 mg.) was treated with dimethyl sulphoxide (15 ml.) in the presence of collidine (0.1 ml.) at 60-80° for 1 hour. The product worked up with ether as usual, giving a syrup (285 mg.). Thin layer chromatography showed a number of spots, the major compounds being cholest-4-ene-3 one, and hydrocarbons; there was no spot corresponding to 3α, 5α -cyclocholestan-6-one. The amount of cholest-4-ene-3 one was determined spectrometrically. The ultraviolet spectrum of the crude product had a maximum at 241 mμ with ε, 8,360 and that of pure cholest-4-ene-3-one had λ241 mμ; ε, 16,600. This indicated that the crude product contained 50 percent of cholest-4-ene-3-one. Separation of 113 mg. of the crude mixture on silica gel chromatoplate, gave cholest-4-ene3-one (80 mg. 55percent), m.p. 82°. The second component consisted of an oily mixture of hydrocarbons (39 mg.) λ cyclohexane 227 mμ (ε, 2310) and which therefore contained little cholestadiene (which has λ 234 mμ; ε, 20,000).

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INVESTIGATION ON AN UNKNOWN GROWTH FACTOR IN COW MILK*

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(Received November 24, 1964)

Introduction

The nutritive value of milk protein has been previously determined by large group of workers by animal experimentation. In the present investigation the microbiological technique of Ford[1,2] has been used to determine the utilisation of cow milk protein by using the organism *Saccharomyces Zymogenes* which, because of its high

*The work was carried out at the Biochemistry Department of the National Institute for Researches in Dairying. Shinfield, Reading, U.K. during a study tour of the author in 1962.

proteolytic character yielded the results of the Relative Nutritive Value (R.N.V.) which agreed well with the Net protein Utilisation Value (NPU) evaluated by animal experiments.[1-3] The present experiment was mainly undertaken to find out if the above organism can grow at equal rate in cow milk medium as well as in the synthetic amino acid medium having equivalent composition as that of milk protein and thus evaluate if the milk contains all the constituents and factors as are necessary for the growth of the above organism.

The technique as developed by Ford [1,2] and adopted by the author[4] in his previous work on the evaluation of the total and available amino acids and Relative Nutritive Value of some fish flours has also been utilised in the present investigation. The various media required for the work were prepared in the following manner.

Medium.—1.—Fresh unpasteurised cow milk was collected from the Dairy Farm of the National Institute for Researches in Dairying, Shinfield, Reading, U.K. early in the morning and 10 ml. of the same were immediately diluted to 500 ml. with distilled water.

Medium 2.—Another 100 ml. sample of the milk was digested in sealed tube with 0.5 ml. of 1 % solution of purified papain (B.D.H.), in citrate buffer of pH 7.6. The tubes were incubated at 16°C. for 3 hours after which the contents were diluted to 500 ml. with distilled water. Digestion with pepsin at pH 2 was not applied in this case as this might coagulate the milk.

Medium 3.—Amino acid mixture equivalent to the protein composition of diluted milk as in (1) was prepared in accordance with the amino acid composition values of cow milk compiled by Henry[5] and Kon.[6] The following is the composition of the mixture dissolved in 1000 ml. of water.

Arginine- 25mg.; Hiitidine- 16mg.; Lysine- 50mg.: Tyrosine-40mg.; Tryptophane-10mg.; Phenylalanine-35mg.; Cystsne-6mg.; Methionine-17mg.; Threonine-31mg.; Serine-32mg.; Leucine-71mg.; Isoleueine-42mg.; Valine-46mg.; Glutamic acid-138mg.; Aspartic acid-33mg.; Glycine-2mg.; Alamine-15mg.; Proline-50mg.

Medium 4.—Half of the above solution i.e. 500 ml. was kept as such and to other half was added the same quantity of papain, as in Medium 2 to equalise the condition of papain digestion. As the amino acids were already in the free condition, actual digestion with papain was not necessary.

Medium 5.—Similar quantity of papain as used in Medii 2 and 4 was diluted with water to 500 ml. This was prepared in order to determine if the protein of papain could effect any growth by auto-digestion by the organism.

In none of the above qreparations extra sodium glutamate, which is an essential factor for the proper growth of the organism, was added as both the milk and the amino acid mixture contained sufficient quantity of glutamic acid. All the medii were adjusted to pH 7.6 before final dilution to 500 ml.

The preparation of the culture tubes with graded doses of the above medii from 0.5 to 8 ml. with addition of 2 ml. basal medium of vitamins and salts, inoculation of the contents of the tubes with *S. Zymogenes*, incubation of the tubes at 37°C. for 48 hours evaluation of the growth response by titration of the acidity after incubation and other details of the technique were the same as adopted by the author [4] in the determination of the total and available amino acids and the Relative Nutritive Value of some fish flours in persuance of the original technique of Ford.[1,2] The titratable acidity values indicating the growth responses against doses are shown in Figure 1.

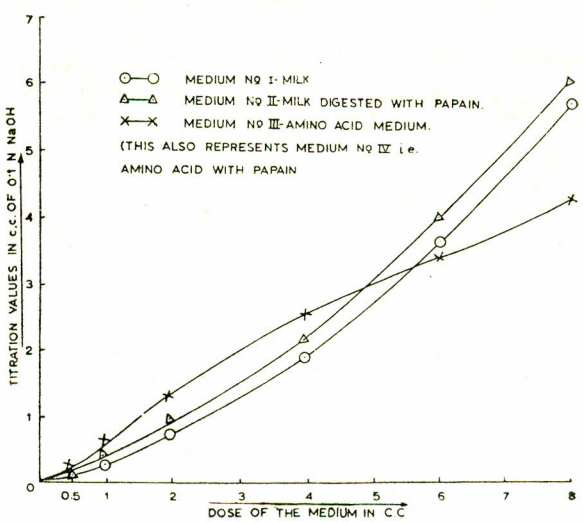

Fig. 1.

The figure shows that the growth response of *S. Zymogenes* in milk alone without papain (Medium 1) is sufficiently pronounced to suggest the high capacity in the organism to digest the milk protein and utilise the liberated amino acids for its growth. Papain digested milk (Medium 2) showed small increase of the rate of growth. The

growth response by amino acid mixture preparation. (Medium 3) was initially higher than milk but the ultimate growth was less than that due to milk alone. As expected, same mixture with added papain (Medium 4) did not produce any additional effect on growth. Similar inactivity of papain (not shown in the figure) was noted in the Medium 5 containing only this enzyme showing that the organism cannot utilise the amino acids of the protein moiety of papain by autodigestion. On the basis of the last experiment it may be inferred that the small increase of growth response by papain-digested milk (Medium 2) is not due to papain but due to some other factor as discussed in the following.

While reviewing the observations of lower growth response by amino acid mixture (Medium 3), having equivalent composition as that of milk protein, one will be led to conclude that the amino acid composition values as described in the texts,[5,6] and on the basis of which the present synthetic amino acid mixture medium had been prepared, have not been correctly determined. But since the standard values compiled by Henry,[5] Kon[6] and others were determined by large group of workers by application of different methods and these also agreed well, there hardly remains any reasons to question the correctness of the above amino acid composition of the milk protein. The only possible explanation which may be offered for higher growth response due to milk alone (Medium 1) is the presence of hitherto unknown factor which may promote growth in *S. Zymogenes* and possibly in other higher organisms also, and that this growth factor remains mainly in the free form and partially forms as bound which is liberated by papain digestion for which some increase of growth occurs due to Medium 2.

It is possible that this growth factor is involved in the metabolism and utilisation of the free amino acids and is partly endogenous in origin i.e. synthesised by the organism and partly available from the external source like milk and thus exogenous in behaviour. This is apparent from the growth curves from which it is noted that the organism in the amino acid Medium 3 and 4 up to the dose level of 4 ml. can easily utilise the free and available amino acids with the help of the endogenous fraction which is already stored in the organism. At higher dose level above 4 ml. there is lag of the growth and this is due to deficiency of the above growth factor, the demand for which can neither be compensated by the quantity of the endogenous fraction of the tissue nor by the amino acid medium, in which the exogenous fraction of the growth factor is absent. This exogenous fraction is, however, available from the milk for which increased growth rates are noted both due to Medii 1 and 2 even at higher dose levels equivalent to those of amino acid Medii 3 and 4.

Similar phenomena of initial increase followed by final decrease in the body weights of rats and other organisms are noted when these are placed under any vitamin deficient dietaries or medium and this is due to initial storage of the vitamins in the tissues which are depleted with the progress of the feeding with the deficient dietaries.

After the completion of the present work the author came across a communication by Davis, *et al.*,[7] who reported the presence of an unknown Chick-Growth Factor in milk and termed this as MFG. The factor extracted from whey was water-soluble, heat-stable, resistant to oxidation and not absorbed by charcoal and anion and cation exchange resins. Whether the growth factor as reported here is the same as the above Chick-Growth factor is yet to be investigated.

Summary

The possibility of the presence of an "Unknown Growth Factor" in cow milk has been discussed by comparative study of the growth response of *Saecharomyces Zymogenesdue* to graded doses of cow's milk against those due to amino acid medium having similar composition as that of the cow's milk.

Acknowledgement.—The author is grateful to Dr. S.K. Kon, Head of the Nutrition Division and to Mr. J.E. Ford of the same Division of National Institute for Researches in Dairying, Shinfield, Reading, U.K. for extending facilities for carrying out this work.

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