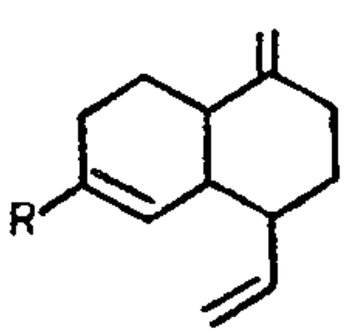
A NEW ACIDIC COMPONENT FROM THE INDIAN VETIVER OIL

In an earlier communication the chemical composition of the Indian Vetiver oil (Punjab variety) was described.[1] The present article reports on the acidic components of the oil.

Vetiver oil isolated by steam distillation of the powdered roots of the plant *Vetiveria zizanioides* Linn. was thoroughly extracted with aqueous potassium hydroxide. Acidification of the alkali extracts afforded a crude acidic product in about 0·2% yield as a dark viscous material. This was reacted with an ethereal solution of diazomethane which gave the corresponding mixture of esters as evidenced from the IR spectrum of the mixture which showed bands at 1,745, 1,700 and around 1,250 cm.$^{-1}$ due to the presence of both saturated and α, β-unsaturated esters. The mixture was purified by passing through a small bed of alumina and subjected to VPC analysis which showed the presence of seven peaks. In confirmation with this observation TLC showed the presence of at least five clearly visible spots. VPC and TLC results taken together showed the presence of one of the components in a large quantity (60%). Attempts were, therefore, made to isolate and characterize this major acidic component of the oil. This acid was isolated in the form of its ester in a pure form. Chemical and spectroscopic data showed that this compound represents the Acid I whose corresponding aldehyde Khusilal II has already been reported to occur as a major component of the Vetiver oil.[2]



IV

I R = COOH
II R = CHO
III R = CH_2OH

All attempts to isolate the components of the mixture of esters by column chromatography over alumina were unsuccessful. However, the chromatography of the esters over silica gel impregnated with silver nitrate succeeded and the above major component was isolated in a state of high purity. It analysed for $C_{15}H_{20}O_2$ (Found : C, 78·10 ; H, 8·84. $C_{15}H_{20}O_2$ requires : C, 77·55 ; H, 8·68%); IR bands at 1,700 and around 1,240 (α, β-unsaturated ester); at 3080, 1640 and 892 (methylenic double bond); at 995 and 915 (vinyl double bond) and bands around 830 cm.$^{-1}$ (trisubstituted double bond). The presence of vinyl double bond showed that this ester has probably the Khusilane carbon skeleton[2] (IV). IR data coupled with its UV spectrum $\lambda_{max.}$ 218 mμ, ϵ, 9,900 confirmed it to be an α, β-unsaturated ester.

Reduction of the ester with LAH afforded a crystalline alcohol which was purified by preparative TLC. It was identified as Khusilol III m.p. and mixed m.p. with an authentic sample 75° and from a comparison of their IR spectra.

Mild alkaline hydrolysis of the ester afforded the corresponding acid (I) m.p. 124°. Mixed m.p. with an authentic sample of the acid remained undepressed. Its IR and UV spectra ($\lambda_{max.}$ 214 mμ, ϵ, 9800) were completely identical with that of the natural product.

Department of Chemistry and Biochemistry,
Punjab Agricultural University,
Ludhiana (India), *March* 28, 1969.

P. S. Kalsi.
I. S. Bhatia.

1. Manchanda, S. K., Bhatia, I. S. and Kalsi, P. S., *Riechstoffe, Aromen, Korperpflegemittel*, 1968, **18**, 415.
2. Kalsi, P. S., Chakravarthi, K. K. and Battacharyya, S. C., *Tetrahedron*, 1964, **20**, 2617.

RETINAL FROM RETRORETINOL

Retroretinol, generally found in deteriorated samples of multivitamin preparations,[1,2] can be prepared by treatment of retinyl acetate with concentrated hydrobromic acid.[3] Barua and Rao[4] reported the formation of a compound whose absorption spectrum resembled that of retroretinol. Although there is indication of *in vivo* conversion of retroretinol to retinol[1,5] there is no report till now of the chemical conversion of retroretinol to retinol. In this communication we report a new procedure for the preparation of retroretinol and the conversion of retroretinol to retinal.

Preparation of Retroretinol.—200 mg. of retinol was treated with an alcoholic solution of dry hydrogen chloride (0·13 N. ; 100 ml.) and allowed to stand for 40 minutes in the dark. The reaction mixture was then neutralised with sodium bicarbonate solution and the product was extracted with light petroleum. Chromatography of the product on water-deactivated alumina (5% water, v/w, resulted

in the separation of four zones. The main greenish-yellow zone containing anhydroretinol[6,7] passed quickly through the column. The second yellow zone was eluted with light petroleum containing 1% (v/v) of ether. The substance from this zone exhibited $\lambda_{max.}$ at 330 mμ (light petroleum). The third yellow zone was eluted with light petroleum containing 5% (v/v) of ether. The substance from this zone showed $\lambda\lambda_{max.}$ at 367, 348 and 332 mμ (solvent : light petroleum). The fourth zone which was eluted, after extrusion, with ether contained a substance that exhibited $\lambda_{max.}$ at 290 mμ in light petroleum.

The substance eluted from the third zone was further purified by chromatography and the pure compound exhibited $\lambda\lambda_{max.}$ 367, 348, 332 mμ with E (1%, 1 cm.) values of 1274, 1593 and 1270, respectively, at the points of maximum absorption in light petroleum. It produced a bluish-violet colour with antimony-trichloride reagent showing $\lambda_{max.}$ at 580 mμ. In the I.R. spectrum it showed band at 3400 cm.$^{-1}$ characteristic of the –OH group. The yield of retroretinol was 10·1%.

The identity of retroretinol prepared as above was further confirmed by preparing retroretinyl acetate. Retroretinol was heated with acetic anhydride in pyridine at 50° C. for 30 minutes, the product was extracted with light petroleum and then chromatographed on a column of water-deactivated alumina (5% water ; v/w). Retroretinyl acetate ($\lambda\lambda_{max.}$ 367, 348 and 332 mμ in light petroleum) was eluted with light petroleum. It produced a violet colour with Carr-Price reagent showing $\lambda_{max.}$ at 575 mμ with a minor maximum at 470 mμ.

The ultra-violet and I.R. spectra and $SbCl_3$ colour absorption maxima of retroretinol and retroretinyl acetate prepared by this new procedure and those prepared by the procedure outlined by Beutel *et al.*[3] were found identical in all respects. Further, co-chromatography of samples of either retroretinol or retroretinyl acetate prepared by these two procedures, on a column of water-deactivated alumina showed that the compounds were identical. Only a single zone was formed on the chromatogram and prolonged development did not effect separation of the zone.

Conversion of Retroretinol into Retinal.—20 mg. of retroretinol obtained by the above procedure was dissolved in 10 ml. of light petroleum and passed through a column of manganese dioxide (300 mg. precipitated, B.D.H.). The filtrate was concentrated and chromatographed on a column of water-deactivated alumina (8% water, v/w). The substance from the main zone which was eluted with light petroleum or better with light petroleum containing ether (1-2%, v/v), was found to be retinal (1·2 mg.). $\lambda_{max.}$ 369 mμ in light petroleum and 380 mμ in ethanol ; $SbCl_3$ colour maximum at 660 mμ.

Retinal thus obtained was found to be identical with an authentic sample prepared from retinol by the method of Ball, Goodwin and Morton[8] in their ultra-violet and $SbCl_3$ colour-visible spectra. Mixed chromatography of the two retinal samples did not result in the separation of zones.

Retinal (10 mg.), prepared from retroretinol, was reduced with $NaBH_4$. The reduced product after chromatographic purification was found to be retinol (7·56 mg.), $\lambda_{max.}$ 325 mμ ; $SbCl_3$ colour $\lambda_{max.}$ 617 mμ. Treatment of retinol with 0·033 N ethanolic hydrogen chloride followed by purification by chromatography gave anhydroretinol, $\lambda\lambda_{max.}$ 390, 369 and 350 mμ ; $SbCl_3$ colour $\lambda_{max.}$ 617 mμ.

Retinal was also obtained in 6% yield when retroretinol, prepared by the procedure of Beutel *et al.*,[3] was oxidised with MnO_2.

Department of Chemistry, K. K. Das.
University of Gauhati, A. B. Barua.
Gauhati-14, N. N. Siddhanta.
Assam, *March* 18, 1969.

1. Varma, T. N. R. and Murray, T. K., *Biochem. Biophys. Acta*, 1963, **78**, 556.
2. —, Erdody, P. and Murray, T. K., *J. Pharm. Pharmacol.*, 1965, **18**, 474.
3. Beutel, R. H., Hinkley, D. F. and Murray, T. K., *J. Am. Chem. Soc.*, 1955, **77**, 5166.
4. Barua, R. K. and Rao, M. V. K., *Analyst*, 1964, **89**, 534.
5. Murray, T. K. and Erdody, P., *Biochim. Biophys. Acta*, 1966, **124**, 190.
6. Edisbury, J. R., Gillam, A. E., Heilbron, I. M. and Mortan, R. A., *Biochem. J.*, 1932, **26**, 1164.
7. Embroe, N. D., *J. Biol. Chem.*, 1939, **128**, 187.
8. Ball, S., Goodwin, T. W. and Morton, R. A., *Biochem. J.*, 1948, **42**, 516.

AFLATOXIN G_1 HEMIACETAL

It has been recently reported[1,2] that aflatoxin-containing extracts from cultures of *Aspergillus flavus* contain two other metabolites apparently related to the aflatoxins, namely, aflatoxin B_{2a} and G_2 Of these the former has been shown to be identical with aflatoxin B_1 hemiacetal obtained by hydration of aflatoxin B_1.[3,4] But no parallel study appears to have been made