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DIASTEREOMERIC EPISULFIDES FROM *EPI*-PROGOITRIN UPON AUTOLYSIS OF CRAMBE SEED MEAL*

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Abstract—When defatted seed meal from *Crambe abyssinica* Hochst ex R. E. Fries is autolyzed, its major thioglucoside, *epi*-progoitrin, undergoes a reaction sequence different from that which occurs upon enzymic hydrolysis of the thioglucoside in an isolated system. The major products are two diastereomeric (2*S*)-1-*cyano*-2-*hydroxy*-3,4-*epithiobutanes*, plus the previously characterized (*S*)-1-cyano-2-hydroxy-3-butene. The isomeric episulfides were structurally characterized primarily through their more stable monoacetyl derivatives. Relationship of the episulfides to (*S*)-1-cyano-2-hydroxy-3-butene, including configuration at the chiral center at carbon 2, was established by desulfurization of the acetate of each isomer with triphenylphosphine to give from both the acetate of (*S*)-1-cyano-2-hydroxy-3-butene. Configurations at the chiral center at carbon 3 have not been established. Formation of these episulfides is most likely due to a nonenzymic reaction that follows initial enzymic formation of a suitable intermediate from *epi*-progoitrin. The diastereomeric episulfides were separated from each other on a column of modified dextran designed for use as a molecular sieve.

INTRODUCTION

PURIFIED *epi*-progoitrin [potassium 2(*S*)-hydroxy-3-butenylglucosinolate] (I) from the seed of *Crambe abyssinica* Hochst ex R. E. Fries (Crambe),[1] when hydrolyzed by mustard thioglucosidase (thioglucoside glucohydrolase 3.2.3.1 as classified by recommendations of the International Union of Biochemistry),[2] yields (*R*)-goitrin (II) and (*S*)-1-cyano-2-hydroxy-3-butene (III).[3]

The same products can be obtained from the *epi*-progoitrin *in situ* in Crambe seed meal by hydrolysis of the meal under selected conditions. The *epi*-progoitrin is freshly harvested or cold-stored seed, however, has a marked tendency to take an alternate pathway of breakdown when the meal is autolyzed. Thus simple moistening of the crushed and defatted seed at room temperature gives an organic aglucon fraction that contains III plus unidentified compounds, but does not contain II.[4] In this paper the isolation and characterization of the diastereomers of (2*S*)-1-cyano-2-hydroxy-3,4-epithiobutane (IV-A and IV-B) from the aglucon fraction are described.

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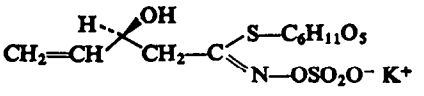
[1] M. E. DAXENBICHLER, C. H. VANETTEN and I. A. WOLFF, *Biochemistry* **4**, 318 (1965).

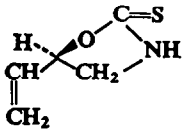
[2] *Comprehensive Biochemistry*, Vol. 13, 2nd edition, p. 142 (edited by M. FLORKIN and E. H. STOTZ). Elsevier, London (1965).

[3] M. E. DAXENBICHLER, C. H. VANETTEN and I. A. WOLFF, *Biochemistry* **5**, 692 (1966).

[4] C. H. VANETTEN, M. E. DAXENBICHLER, J. E. PETERS and H. L. TOOKEY, *J. Agr. Food Chem.* **14**, 426 (1966).



I



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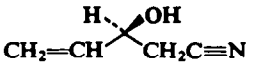
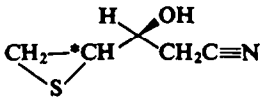


III



IV, Isomers A and B

RESULTS AND DISCUSSION

For a typical preparation, the aglucon fraction was 1.3 per cent of the starting meal and contained 16–17 per cent S, 10·5–11 per cent N, and 65–70 per cent organic nitrile, measured by quantitative i.r. spectroscopy and calculated as III.[3] At least 15 per cent of the fraction was shown to be III.[4]

Comparison of an i.r. spectrum for the fraction with the spectrum for pure III showed many similarities: a broad intense band about 3450 cm^{-1} (hydroxyl); absorption in C–H stretch region of comparable intensity (weak) although of slightly different fine structure; medium intensity band near 2260 cm^{-1} (nitrile); 1420 cm^{-1} (strong); 1125 cm^{-1} (medium); 1055 cm^{-1} (strong from both but broader from the mixture); 990 cm^{-1} and 935 cm^{-1} (vinyl doublet weaker from the mixture); 880 cm^{-1}; 850 cm^{-1} and 810 cm^{-1} (broad bands of weak intensity). The most readily observed differences between the two i.r. spectra were a weak to medium intensity absorption at 620–640 cm^{-1}, assigned to C–S stretch, and some absorption of weak intensity at 1630–1700 cm^{-1} from the mixture, believed caused by unknown extraneous materials. Also, the relative intensities of several of the bands common to both spectra were different.

The mixture gave a negative test with Grote's reagent,[5] even after addition of KCN, which indicated that the sulfur was not present as a thiol, thione, or disulfide.

An NMR spectrum of the mixture showed the absence of C-methyl protons and when compared to the spectrum from III[3] the vinyl group proton absorption pattern δ5·1–6·3 was of diminished relative intensity and there was greater complexity from δ2·4–4·1.

Descending paper chromatography of the fraction on Whatman* No. 1 paper with *n*-butanol–ethanol–water (4:1:4) upper phase, and detection either with alkaline silver or with platinum and potassium iodide reagent,[6] gave an elongated spot near the solvent front. This spot was later shown to be due to IV-A and IV-B by paper chromatography of the isolated compounds. Thin-layer chromatography (TLC) on Silica Gel G in several solvent systems, with iodine vapor or with sulfuric acid–dichromate char detection, indicated three

* The mention of firm names or trade products does not imply that they are endorsed or recommended by the Department of Agriculture over other firms or similar products not mentioned.

[5] I. W. GROTE, *J. Biol. Chem.* **93**, 25 (1931).

[6] G. TOENNIES and J. J. KOLB, *Anal. Chem.* **23**, 823 (1951).

major components in the mixture. However, no solvent system was found for TLC that effectively separated all three.

Separation of the components was subsequently achieved on a column of modified dextran.

Figure 1 shows a typical weight distribution curve from elution of the components from the column. From 63 to 80 per cent of the material put on the column was recovered as the three isolated compounds in the weight ratio of 1:1:1·8. Weight recovery of a known amount of separated IV-B when rechromatographed was 75 per cent. Loss of the isolated compounds is likely due both to volatilization of III and to instability of IV-A and IV-B. Unidentified substances present in the fraction in smaller amounts gave an odor reminiscent of mercaptans and hydrogen sulfide. Isolated III, IV-A, and IV-B had no detectable odor.

From a typical separation the appropriate fractions of IV-A were combined on the basis of the weight curve (Fig. 1) to give 44 mg of clear oil, n_D^{20} 1·531. (Found: C, 46·2; H, 5·6; N, 11·1; S, 24·1; M.W., 142. C_5H_7NOS required: C, 46·4; H, 5·4; N, 10·9; S, 24·8 per cent; M.W., 129.)

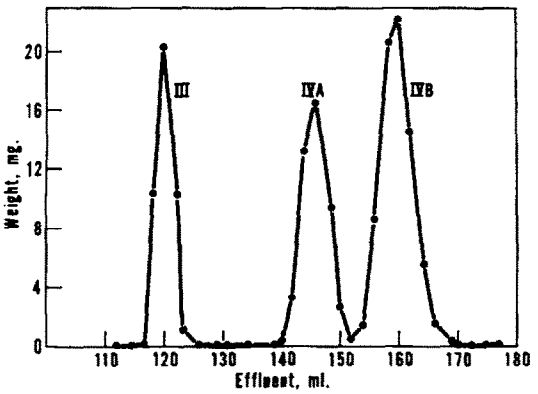


FIG. 1. COLUMN SEPARATION ON MODIFIED DEXTRAN OF THE NITRILE MIXTURE FROM AUTOLYZED CRAMBE MEAL.

An i.r. spectrum of the oil included absorption (cm^{-1}) as follows: 3440, 2260, 1420, 1125, 1073 and 1050, 990 and 937 (weak), and 640 (C–S stretch).

The u.v. spectrum at 220–280 nm showed a progressive increase in absorption as measured from the longer to the shorter wavelengths with a shoulder-type maximum occurring at 255 nm in ethanol, $\epsilon = 66$. For the NMR spectrum see Fig. 2.

The fractions combined for IV-B based on the weight curve (Fig. 1) gave 80 mg of clear oil, n_D^{20} 1·529. (Found: C, 46·3; H, 5·5; N, 10·8; S, 24·0; M.W., 141. C_5H_7NOS required: C, 46·4; H, 5·4; N, 10·9; S, 24·8 per cent; M.W., 129.)

An i.r. spectrum of the oil appeared substantially the same as that for IV-A. However, the 1073 and 1050 cm^{-1} bands were essentially one broad band with the maximum near 1060 cm^{-1} and instead of the absorption at 640 cm^{-1} as from IV-A, the absorption was at 620 cm^{-1}. U.v. analysis at 220–280 nm gave the same shoulder-type maximum absorption as IV-A with the maximum at 257 nm in ethanol, $\epsilon = 91$. The NMR spectrum in deuteriochloroform was the same as that of IV-A (Fig. 2) except for minor differences in chemical shift values.

IV-A and IV-B easily underwent apparent polymerization unless kept in a solvent at low temperature (< 5°). Acetylation of their hydroxyl function improved stability. Accordingly, the more stable monoacetyl derivatives were prepared for further characterization work.

The acetate of III has n_D^{20} 1·4385, ECL = 12·7 (LAC-2-R 446 polyester, 200°, with saturated fatty acid methyl esters used as standards.)[7] An i.r. spectrum showed absorption (cm^{-1}) including 2260 (C≡N), 1740 (ester carbonyl strong), 1650, 1420, 1370, 1235 (strong), 1108, 1030, and 993 and 943 (vinyl) with absence of absorption 3400–3500 as evidence that the hydroxyl function was esterified. An NMR spectrum in deuteriochloroform showed the acetoxy protons near δ2·1 and a doublet for the methylene protons near δ2·7. The single proton on the acetate-bearing carbon came under the absorption pattern of the vinyl group protons δ5·1–6·0 which integrated for 4 protons. ORD measurements (Fig. 3) gave a plain positive curve similar to that of III.[3]

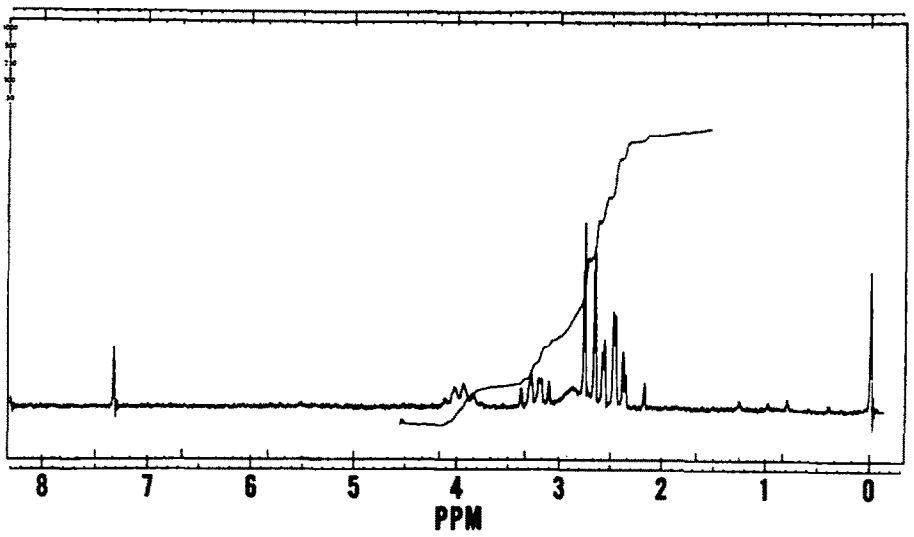


FIG. 2. NMR SPECTRUM OF IV-A IN $CDCl_3$; INTERNAL STANDARD: $(CH_3)_4Si$.

For the acetate of IV-A; n_D^{20} 1·494; ECL = 19·1. (Found: C, 48·6; H, 5·3; N, 8·2; S, 18·0; M.W. 176–180. $C_7H_9O_2NS$ required: C, 49·1; H, 5·3; N, 8·2; S, 18·7 per cent; M.W., 171.)

U.v. absorption in ethanol gave a shoulder-type maximum at 254 nm as did IV-A. An i.r. spectrum included absorption (cm^{-1}) as follows: absence of absorption 3400–3500 (no hydroxyl), 2260 (nitrile), 1745 (ester carbonyl, strong), 1420, 1370, 1230, 1040 and 1053 doublet, and 640 (C–S stretch).

The NMR spectrum in deuteriochloroform (Fig. 4) shows the three acetoxy protons δ2·1; four closely spaced doublets (2 protons) with the multiplet centered near δ2·5 for the protons on the terminal carbon of the episulfide group; the single proton multiplet δ3·2 for the single proton on the other carbon of the episulfide group; and a 2 × 3 multiplet centered δ4·8 (1 proton) for the proton on the carbon bearing the acetate group.

For the acetate of IV-B; n_D^{20} 1·491; ECL = 18·3. (Found: C, 49·0; H, 5·3; N, 8·2; S, 17·9; M.W. 194. $C_7H_9O_2NS$ required: C, 49·1; H, 5·3; N, 8·2; S, 18·7 per cent; M.W., 171.)

[7] T. K. MIWA, K. L. MIKOLAJCZAK, F. R. EARLE and I. A. WOLFF, *Anal. Chem.* 32, 1739 (1960).

U.v. absorption in ethanol gave a shoulder-type maximum at 257 nm as did IV-B. An i.r. spectrum of the oil included absorption as follows: no significant absorption 3400–3500 cm^{-1} (no hydroxyl), 2260 (nitrile), 1745 (ester carbonyl, strong), 1420, 1370, 1225 (broad), 1033 broad (instead of 1040 and 1053 doublet from IV-A acetate), 635 and 620 (C–S stretch).

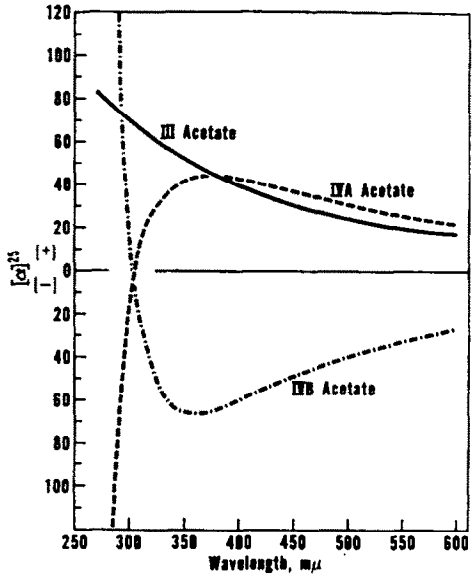


FIG. 3. ORD CURVES (*c.* 0·5–1·0, METHANOL) OF ACETYLATED NITRILES.

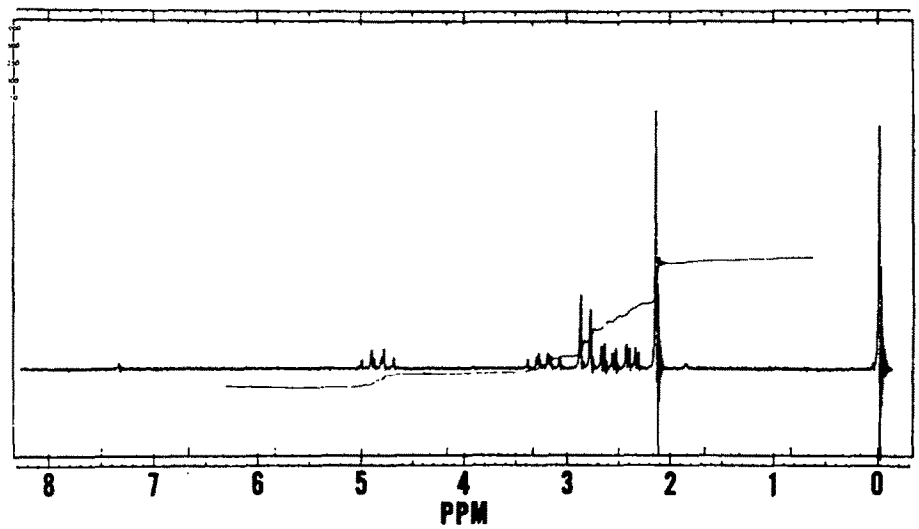


FIG. 4. NMR SPECTRUM OF ACETATE FROM IV-A IN $CDCl_3$; INTERNAL STANDARD: $(CH_3)_4Si$.

An NMR spectrum in deuteriochloroform (Fig. 5) shows the acetoxy protons absorption δ2·15; three closely spaced doublets δ2·55 (center doublet) for the terminal episulfide group protons; a doublet (2 protons) for the methylene group near δ2·9; a multiplet (1 proton) for

the single proton on the other episulfide carbon centered near δ3·15; and a multiplet with 2 × 3 multiplicity near δ4·65 for the proton on the carbon bearing the acetate group.

The ORD curves for the acetates of IV-A and IV-B are given in Fig. 3. Compared to the ORD curves from the acetylated IV-A and IV-B, the curves for the corresponding unacetylated materials (not illustrated) were similar in shape and appropriate in sign but of greater magnitude in specific rotation.

Desulfurization of the acetates of IV-A and IV-B to give the acetate of III was accomplished with triphenylphosphine. Triphenylphosphine is known to react with episulfide groups to form a double bond.[8] Formation of the acetate of III from the acetates of both IV-A and IV-B by reaction with triphenylphosphine is additional evidence that the episulfide is between carbon 3 and 4. Furthermore, since the products formed from each have an ORD curve identical to the acetate of III (Fig. 3), with (*S*) configuration about its chiral

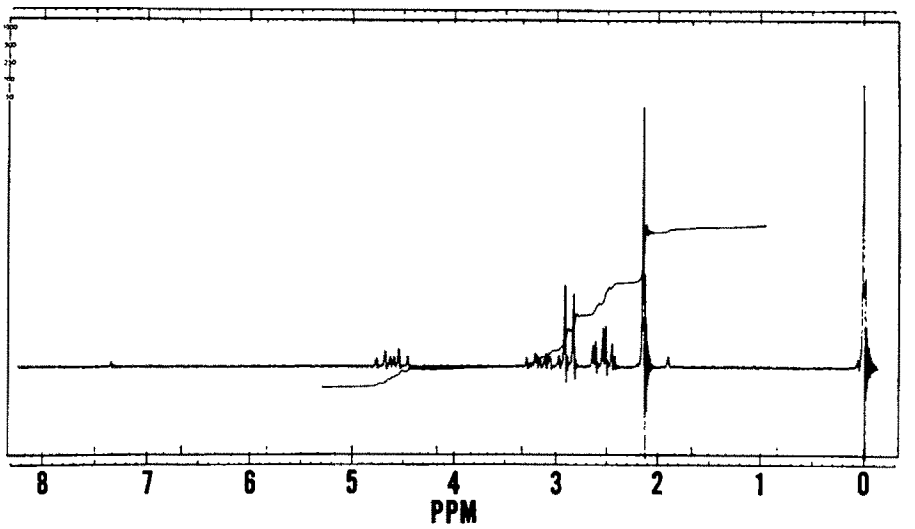


FIG. 5. NMR SPECTRUM OF ACETATE FROM IV-B IN $CDCl_3$; INTERNAL STANDARD: $(CH_3)_4Si$.

center, the chiral center at carbon 2 in both IV-A and IV-B must also have the (*S*) configuration. Although absolute configuration about the chiral center at carbon 3 in IV-A and IV-B was not established, a postulate has been made based on optical rotation data.[9]

Structures proposed for IV-A and IV-B are therefore based on elemental analysis and molecular weight; u.v., i.r., and NMR spectra; ORD measurements of the isolated compounds and their monoacetate derivatives and desulfurization of both episulfide-containing acetates to the corresponding known olefinic acetate.

U.v. absorption with a maximum at 254–257 nm of low absorptivity is in agreement with literature reports for other episulfide compounds.[10,11] I.r. measurements of the isolated components showed typical absorption for hydroxyl and nitrile functional groups and absorption at 620–640 cm^{-1} that could be assigned to C–S stretch.[12]

[8] R. E. DAVIS, *J. Org. Chem.* **23**, 1767 (1958).
[9] M. E. DAXENBICHLER, C. H. VANETTEN, W. H. TALLENT and I. A. WOLFF, *Can. J. Chem.* **17**, 1971 (1967).
[10] R. E. DAVIS, *J. Org. Chem.* **23**, 216, 1380 (1958).
[11] H. P. KAUFMANN and R. SCHICKEL, *Fette, Seifen, Anstrichmittel* **65**, 625 (1963).
[12] G. B. GUTHRIE, D. W. SCOTT and G. WADDINGTON, *J. Am. Chem. Soc.* **74**, 2795 (1952).

The NMR spectra for IV-A and IV-B and their respective acetates were consistent with the proposed structures. Only minor differences between the diastereoisomeric forms could be observed in the spectra.

Since the reaction involved in the formation of the episulfides was not stereospecific, their formation is likely due to a nonenzymic reaction which occurred after enzymic hydrolysis of the thioglucoside.

During autolysis of a rapeseed (*Brassica napus* L.), which contains progoitrin, we find that the antipodes of II, III, IV-A, and IV-B are products of the thioglucoside hydrolysis.[9] Thus, the formation of episulfide-containing nitriles is not unique to Crambe seed. It would be of interest to know if episulfides are formed from gluconapin found in *B. napus* which yields butenyl isothiocyanate.[13]

The diverse nature of natural thioglucosides with respect to the organic aglucon part of the molecule has been extensively developed by Kjaer and coworkers.[14] The formation of nitriles, as well as isothiocyanates, as possible hydrolysis products was difficult to explain before revision of the basic structure of these thioglucosides by Ettlinger and Lundeen.[15] Virtanen and coworkers[16] reported the formation of benzyl isothiocyanate, benzyl thiocyanate, and benzyl cyanide from glucotropaeolin when seed of *Lepidium sativum* are moistened. From their results, as well as ours, the end-products from natural thioglucoside hydrolysis, especially in autolysis of a plant material containing them, are more diverse than originally thought. Biological effect is likely to be quite varied when such plants are used for feed or food. Such variation may depend on even minor differences in preparation[4] as well as the specific thioglucosides that are present.

EXPERIMENTAL

Preparation and Description of Aglucon Products

The aglucon fraction was prepared as previously described,[4] which consisted of aqueous acetone extraction of autolyzed Crambe meal followed by isolation of aglucon products by extraction into ethyl ether. A useful modification of this procedure, used in most of the current work, consists of extraction of the aglucon products from the autolyzed meal with hot water. The hot-water extract is then treated with two volumes of ethanol which precipitates materials which cause emulsions. The precipitate is removed by decantation and filtration after which the ethanol is removed *in vacuo* on a rotatory evaporator. The aglucon products are extracted from the residual aqueous solution into ethyl ether as described before.[4] The products obtained by either extraction procedure are similar in quantity and composition.

Column Chromatography on a Modified Dextran

Separation of III, IV-A, and IV-B from the aglucon-products fraction was achieved on a column (1·3 × 109 cm) of 40–120 μ Sephadex G-10 (Pharmacia, Uppsala, Sweden) prepared as described by Flodin.[17] From 0·7 to 1·0 g of the fraction was dispersed in 5 ml of water and centrifuged. From 1·0 to 1·4 ml of the supernatant was applied to the column as described by Flodin. A similar volume of the supernate was taken to constant weight by removal of the water in a desiccator for estimation of the sample size, which was about 200 mg. The column was operated at 26 to 28° with water as the elutriant. A uniform flow rate of 4·0 to 4·5 ml per hr was maintained with a pump (Model CHMMI-A-29-R, Mini Pump, Milton Roy Co., Philadelphia). The effluent, after the void volume of about 50 ml, was collected in fractions of 2·0 to 2·25 ml. Each fraction was extracted with three 4-ml volumes of ethyl ether. The ether in tared 30-ml beakers was evaporated at room temperature and the residue dried over calcium sulfate.

[13] A. KJAER, J. CONTI and K. A. JENSEN, *Acta Chem. Scand.* 7, 1276 (1953).
[14] A. KJAER, *Progr. Chem. Org. Nat. Prod.* **18**, 122 (1960).
[15] M. G. ETTLINGER and A. J. LUNDEEN, *J. Am. Chem. Soc.* **78**, 4172 (1956).
[16] A. I. VIRTANEN, *Phytochem.* 4, 207 (1965).
[17] P. FLODIN, *Dextran Gels and Their Application in Gel Filtration*, 3rd edition, 85 pp. Meijels Bokindustri, Halmstad (Pharmacia, Uppsala) (1963).

Preparation of Acetylated Nitriles

Separated III, IV-A, and IV-B were acetylated as follows: At 0 to 5° a 100-mg sample was added to 0·25 ml of pyridine:acetic anhydride (1:1 v/v). The reaction mixture was kept near 0° for 16 hr. A column (1 × 18 cm) of 12 g silica gel 60–100 mesh (Adsorbosil CAB 6010, Applied Science Laboratories, State College, Pa.) was prepared by pouring the adsorbent as received in the glass column. The reaction mixture was applied to the top of the column followed by a rinse of the container with *ca.* 0·25 ml $CHCl_3$. After the top of the column was covered with a glass-wool plug, elution was immediately started with $CHCl_3$:ethyl acetate:methanol (15:4:1). The acetate derivatives followed closely behind the solvent front and were readily separated from the reagents and other material. In practice, small volume (0·5–1·0 ml) fractions were collected until the odor of pyridine was noted. The appropriate fractions for the acetate(s) were then combined on the basis of TLC on Silica Gel G with the same solvent mixture used for the column elution or on the basis of GLC analysis. Care was taken to exclude fractions that contained pyridine. Yields were 70–90 per cent of theory.

The acetate of IV-A was also prepared by acetylation (as above) of the entire aglucon fraction and separation from the mixture of acetates by column chromatography or by TLC. Thus for one such preparation, 800 mg of the acetate mixture (free of acetic anhydride and pyridine) was chromatographed on a column (3 × 30 cm) prepared from 130 g Adsorbosil. The column was prewashed with 100 ml ethyl ether : light petroleum (75:25) followed by 100 ml ether:light petroleum (25:75). The sample was put on the column followed by 1 ml ethyl ether rinse and covered with a layer of glass wool. The column was developed first with 100 ml of ether:light petroleum (25:75) followed by a solvent system in which the ethyl ether was progressively increased to a 50:50 composition. The acetate of IV-A eluted last and was free of other components. Acetate IV-A was also separated by TLC on Silica Gel G with ether:hexane (60:40) as the solvent. By both methods the acetates of III and IV-B migrated together.

Deacetylation of Acetate IV-B

To 216 mg of acetate IV-B was added 6 ml of ethanol saturated with ammonia. The solution was held at 0–5° overnight. After removal of most of the solvent with a jet of N_2, the residue was dissolved in 1 ml of water and centrifuged to remove undissolved material. The formation of IV-B through removal of the acetate group was demonstrated by TLC and GLC. The supernatant was applied to the Sephadex column described above. 21 mg (13 per cent of theory) was recovered as IV-B at the expected elution volume position. An i.r. spectrum of the isolated material agreed with that obtained previously for IV-B.

Desulfurization of Acetates of IV-A and of IV-B

To 140 mg of IV-A in 1 ml of benzene was added 220 mg of triphenylphosphine. The reaction mixture was heated at gentle reflux about 3 hr. GLC of a sample gave an excellent peak for acetate III and the acetate of IV-A was not detected. After removal of the benzene with a jet of N_2, the residue was triturated three times with ethyl ether. The combined triturates were evaporated and the product was dissolved in 0·5 ml $CHCl_3$ and applied to a 100-g column (3 × 22 cm) of Adsorbosil and eluted with ethyl ether:light petroleum (1:1). Flow rate was about 2 ml/min; collections were made in 10-ml fractions. On the basis of GLC and TLC fractions No. 19 through 34 were combined to yield 53 mg (47 per cent of theory). This reaction product was identical to the acetate of III by TLC, GLC, i.r., NMR, and ORD.

In the same manner, the triphenylphosphine reaction and separation were carried out with IV-B acetate. Reaction of 134 mg of acetylated IV-B and 215 mg triphenylphosphine gave 40 mg (37 per cent of theory). As with the acetate of IV-A the triphenylphosphine reaction product was identical to the acetate of III by TLC, GLC, i.r., NMR, and ORD.

All i.r. spectra were taken as neat liquids between KBr disks.

Instruments used for physical measurements were: for u.v., a Beckman Model DK-2A spectrophotometer; for i.r., a Perkin-Elmer Corp. Model 137B or Model 337; for NMR, a Varian Associates Model A60 NMR spectrometer; for ORD, a Cary Model 60 recording spectropolarimeter; for molecular weights, a Mechrolab Inc. vapor pressure osmometer Model 301A; and for GLC, a Burrell Kromotog Model K-5 equipped with a thermal conductivity detector and a 200 × 0·6 cm glass column.

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