5. *Sporidesmins. Part III.*[1] *The Degradation of Sporidesmins under Anhydrous Acid Conditions.*

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When acetylated sporidesmins were treated with anhydrous acid the product was a C_{18} indole derivative (V) whose structure has been deduced by chemical degradation and mass spectrometry. The dioxopiperazine from α-methylaminoacrylic acid and the enol acetate of α-formylglycine was isolated in very low yield as a by-product of the reaction of sporidesmin diacetate (I) with zinc and acetic acid.

The degradation of sporidesmin to "anhydrodethiosporidesmin" (V) and its transformation products were reported in a preliminary note [2] concerning the structure of the metabolite. This work and related topics are reported in detail in this paper.

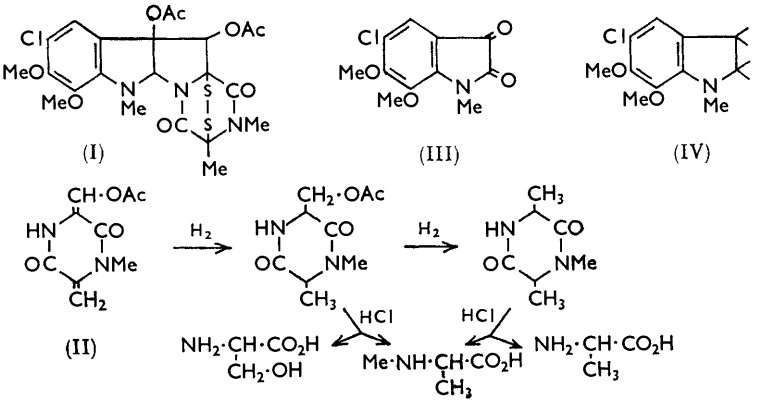
When sporidesmin diacetate [3] (I) was treated with zinc and acetic acid it was sometimes possible to isolate in low yield a monoenol acetate, $C_9H_{10}N_2O_4$ (II), which gave methylamine [4] and traces of serine on acid hydrolysis. The presence of a single replaceable hydrogen atom, possibly of an NH group ($\nu_{max.}$ 3300 and 3100 cm.$^{-1}$), was shown by mass spectrometry, for both the parent peak (*m/e* 210) and the fragment peaks at *m/e* 140, 139 were increased by one unit after deuteration. Catalytic hydrogenation (*ca.* 3 mol.) gave a product which was not purified though its infrared spectrum showed no bands assignable to an enol acetate (1775 cm.$^{-1}$) or a methylene group (1605 cm.$^{-1}$) and which suggested that it was related to dioxopiperazine. Hydrolysis of the hydrogenation product gave *N*-methylalanine, alanine, and serine in the proportion 10 : 7 : 3.[5] The isolation of serine as well as alanine indicates that hydrogenolysis occurs after saturation of the enol-acetate group, thus indicating the allylic nature of the intermediate methylene compound. The results given above can only be interpreted by assuming structure (II) for the C_9 enol acetate.

The main product isolated after reaction of sporidesmin diacetate (I) and anhydrous acid was a neutral yellow indole, $C_{18}H_{16}ClN_3O_4$ (V) ("anhydrodethiosporidesmin" [2]). No O–H or N–H stretching band was observed in its infrared spectrum. A band at 1595 cm.$^{-1}$ suggested the presence of a $>C=CH_2$ group and the proton doublet at τ 3·96 and 4·84 in the nuclear magnetic resonance (n.m.r.) spectrum support this view. A comparison of its ultraviolet spectrum with that of its dihydro-derivative (VIII, see below) indicated that the $>C=CH_2$ group was conjugated with the chromophore of the molecule.

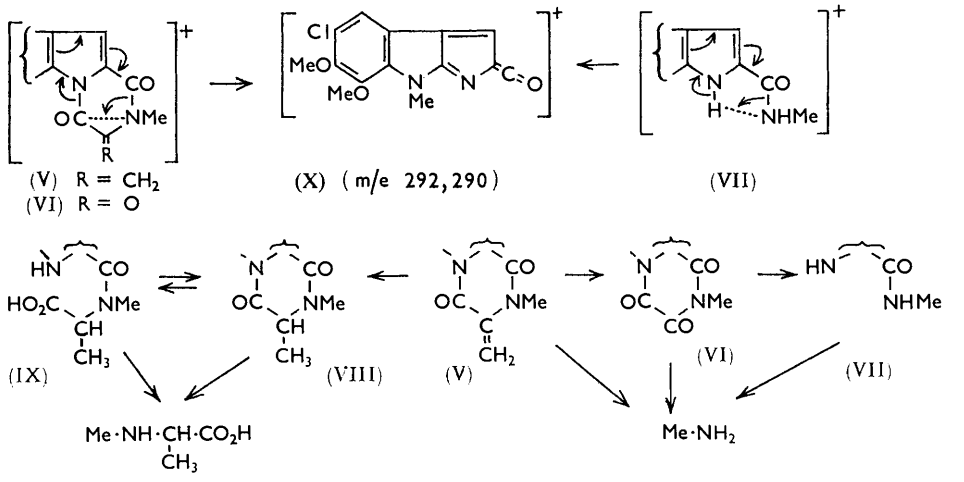
The isatin (III) [1] was obtained when the C_{18} indole (V) was oxidised with aged manganese dioxide: similarly, the 4-nitro-derivative of this isatin was obtained after treatment with nitric acid. The fragment (IV) is therefore present in the yellow indole. Oxidation with freshly prepared manganese dioxide provided, by contrast, a red indole, $C_{17}H_{14}ClN_3O_5$

[1] Part II, Hodges, Ronaldson, Taylor, and White, *J.*, 1963, 5332.
[2] Hodges, Ronaldson, Taylor, and White, *Chem. and Ind.*, 1963, 42.
[3] Ronaldson, Taylor, White, and Abraham, *J.*, 1963, 3172.
[4] Atkinson and Taylor, *J.*, 1955, 4241.
[5] Russell, Synge, Taylor, and White, *J.*, 1962, 554.

(VI), whose ultraviolet spectrum was very similar to that of its yellow parent, and like the latter it gave methylamine and no amino-acids on vigorous acid hydrolysis. Both indoles (V and VI) on mild alkaline treatment gave the same methylamide, $C_{15}H_{16}ClN_3O_3$ (VII).



The C_{18} yellow indole (V) was readily converted into a dihydro-derivative (VIII) by catalytic hydrogenation or by reduction with zinc and acetic acid. The 4-proton triplet at τ 7·95, 8·32, and 8·45, absent from the n.m.r. spectrum of the yellow indole (V), is plausibly assigned to a $CH{-}CH_3$ group, and the presence of such an entity was confirmed when a high yield of *N*-methylalanine was obtained on acid hydrolysis (no methylamine was obtained by this reaction). Brief treatment of the dihydro-derivative (VIII) with alkali gave an acid $C_{18}H_{20}ClN_3O_5$ (IX) which was easily converted back into the dihydro-compound (VIII by acetic anhydride and sodium acetate. The ultraviolet spectrum of the dihydro-compound (VIII) was very similar to that of the C_{15} methylamide (VII) showing that the main chromophore of the intermediates in the transformations described above was unaffected.

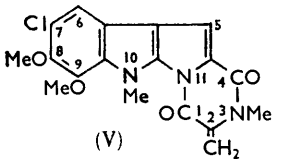


This conclusion received considerable support from an examination of the mass spectra of the yellow indole (V), its C_{17} oxidation product (VI), and the C_{15} methylamide (VII). These three compounds all provided main fragment ions of masses 292, 290; 277, 275; and 234, 232. The ratio of the peak height of each pair was equal to the ratio of the isotopic abundances $^{37}Cl/^{35}Cl$, indicating the presence of one chlorine atom in each ion.[6] When the

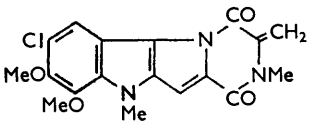
[6] Beynon, " Mass Spectrometry and its Applications to Organic Chemistry," Elsevier, Amsterdam, 1960, p. 299.

C_{15} methylamide (VII) was deuterated in the inlet system of the spectrometer [7] the ion-radicals of mass 292, 290 (and also those of mass 277, 275 and 234, 232) were still observed, although the mass of the parent ion (*m/e* 323, 321) was increased by two units (to *m/e* 325, 323). In addition, evidence for the presence of a third, less readily exchanged proton (similar to a proton occupying the β-position of an indole or pyrrole) was obtained during the deuteration experiments. These results confirm the presence of a methylamide because the expulsion of a neutral fragment of mass 31, having two exchangeable protons, must occur in the formation of the ion-radicals of *m/e* 292, 290. They also suggest that the structure (X) can be assigned to this ion, which may be formed by the mechanisms illustrated. The degradation chemistry of the yellow indole (V) is summarised in the scheme, and thus the presence of the portion $>N{\cdot}CO{\cdot}C(:CH_2){\cdot}NMe{\cdot}CO{\cdot}$ is established.

It will be noted that an identical fragment is found in the C_9 enol acetate (II). The latter and the isatin (III) account for all the carbon and nitrogen atoms present in the yellow indole (V). The evidence presented above shows that the group $-CO{\cdot}C(:CH_2){\cdot}NMe-$ is not bonded to the atoms in the indole ring system. It thus seems probable that the isatin and the C_9 enol acetate represent non-coincident fragments of the C_{18} compound (V). Thus an expression for the yellow indole can be obtained by fusing these two entities together. There are only two ways of doing this that are consistent with its chemistry



(V)



(XI)

and of these two possibilities (V and XI), only (V) is acceptable [8] if no skeletal rearrangement occurs in the formation of the yellow indole from sporidesmin diacetate.

Experimental

Infrared and ultraviolet spectra were measured on Perkin-Elmer " 137 " spectrophotometers. Radiofrequency spectra were determined on Varian instruments, and unless otherwise stated, for deuterochloroform solutions. Mass spectra were determined on an Atlas CH4 instrument.

Action of Zinc and Acetic Acid on Sporidesmin Diacetate.—Sporidesmin diacetate (3·05 g.), glacial acetic acid (30 ml.), acetic anhydride (3 ml.), and " AnalaR " granulated zinc (15 g.) were stirred under reflux for 2 hr., cooled, and diluted with chloroform until most of the precipitate dissolved. The mixture was filtered and evaporated. The residue was digested with several portions of hot benzene until the digests were no longer yellow, and the combined extracts were run on to silica gel [3] (125 g.; 26 × 4·5 cm.). Sulphur (100 mg., 30%) was eluted with benzene (500 ml.), and sporidesmin diacetate (50 mg.) with 9 : 1 benzene–ether (500 ml.). A yellow band (950 mg.; m. p. 240—252°) was then eluted with 4 : 1 benzene–ether (1 l.) and recrystallised from n-butyl alcohol (150 ml.) as needles (550 mg.), m. p. 263—264°. *7-Chloro-1,2,4,10-tetrahydro-8,9-dimethoxy-3,10-dimethyl-2-methylene-1,4-dioxo-3H-pyrazino[1′,2′ : 1,5]-pyrrolo[2,3-b]indole* separated from chloroform–methanol as yellow needles (530 mg.), m. p. 269—270° (Found: C, 57·35; H, 4·45; Cl, 9·6; N, 11·3; O, 17·4; OMe, 15·9; *N*-Me, 7·2; $Me{\cdot}NH_2$ on hydrolysis,[3,4] 7·8. $C_{18}H_{16}ClN_3O_4$ requires C, 57·85; H, 4·3; Cl, 9·5; N, 11·25; O, 17·1; 2OMe, 16·6; 2*N*-Me, 8·0; $Me{\cdot}NH_2$, 8·0%), *m/e* 375, 373; 292, 290; 277, 275; 234, and 232, τ 2·35, 2·59 (2 aromatic protons), 3·96 (doublet), 4·84 (doublet), 5·59 (intensity 3, *N*-CH_3), 6·71 (intensity 3, *N*-CH_3), 5·98 (intensity 3, *O*-CH_3), and 6·07 (intensity 3, *O*-CH_3), $\nu_{max.}$ (in $CHCl_3$) 1710, 1655, and 1595 $cm.^{-1}$, $\lambda_{max.}$ (in MeOH) 258, 297, and 394 mμ (log ε 4·42, 4·50, and 3·82).

Sometimes, on a smaller scale (940 mg. of sporidesmin diacetate), the solution was red rather than yellow at the end of the reaction. Such solutions, when worked up as described above,

[7] Shannon, *Tetrahedron Letters*, **1963**, 801.
[8] Fridrichsons and Mathieson, *Tetrahedron Letters*, 1962, 1229.

provided on further elution of the column with benzene–ether (4 : 1) the dihydro-derivative (VIII) (18 mg.) of the above indole (see below), followed by the C_9 ester (II), which was purified by sublimation at 220°/0·1 mm. *3-Acetoxymethylene-1-methyl-6-methylene-2,5-dioxopiperazine*, crystallised from ethyl acetate–chloroform, had m. p. *ca.* 245° (decomp.) (Found: C, 51·2; H, 5·55; O, 31·1; Me·NH_2 on hydrolysis,[3,4] 14·5%; Equiv.,[9] 140. $C_9H_{10}N_2O_4$ requires C, 51·4; H, 4·8; O, 30·45; Me·NH_2, 14·75%; Equiv., 210), *m/e* 210, 168 (base peak), 140, 139, 134 (metastable) (when deuterated in the inlet system of the spectrometer,[7] 211, 141, 140), $\lambda_{max.}$ (in MeOH) 282 mμ (log ε 4·33), $\nu_{max.}$ (in paraffin) 3300, 3100, 1645, and 1605 cm.$^{-1}$. The yellow indole (V) was also isolated from the reactions listed in the annexed Table.

Sporidesmin derivative	Reagent	Time (hr.)	Temp.	Yield (%) of (V)
Diacetate (I)	BF_3–Et_2O	48	20°	63
,, ...	AcOH	2	120°	40
(Sporidesmin)	P_2O_5–C_6H_6	48	Room temp.	<1
Sporidesmin-B[3] acetate	Zn–AcOH	2	120°	9

Reduction of the Yellow Indole (V).—The indole (V) (0·5 g.), acetic acid (50 ml.), and zinc dust (3 g.) were heated together under reflux until the yellow solution was almost colourless (the time of reaction depended on the state of subdivision of the zinc). The mixture was diluted with ether, filtered, washed with water, and evaporated. The residue in benzene was adsorbed on silica gel. Elution with benzene–ether (4 : 1) gave *7-chloro-1,2,4,10-tetrahydro-8,9-dimethoxy-2,3,10-trimethyl-1,4-dioxo-3H-pyrazino*[1′,2′ : 1,5]*pyrrolo*[2,3-*b*]*indole* as colourless prisms (from acetone) (385 mg.), m. p. 196—198° (Found: C, 57·35; H, 4·9. $C_{18}H_{18}ClN_3O_4$ requires C, 57·55; H, 4·85%), $\lambda_{max.}$ (in MeOH) 243, 282, and 335 mμ (log ε 4·37, 4·31, and 4·37), $\nu_{max.}$ (in CCl_4) 1720 and 1655 cm.$^{-1}$, τ (in pyridine) 5·48 (intensity 3), 5·90 (intensity 6), 6·79 (intensity 3), and 7·95, 8·32, 8·45 (triplet, intensity 4). The same compound was obtained from the indole (V), but in lower yield, by hydrogenation at 20°/760 mm. over 5% palladium–charcoal in ethyl acetate.

5-Chloro-6,7-dimethoxy-1-methyl-4-nitroisatin.—The indole (V) (31 mg.) and nitric acid (*d* 1·42; 1 ml.) were heated together at 60° for 5 min., then diluted with water, and the benzene-soluble products were adsorbed on silica gel. A red band eluted with benzene–ether (99 : 1) gave *5-chloro-6,7-dimethoxy-1-methyl-4-nitroisatin* separating from carbon tetrachloride as red needles (4·9 mg.), m. p. 162—163° (Found: C, 43·65; H, 3·3; N, 9·5; O, 31·75. $C_{11}H_9ClN_2O_6$ requires C, 43·95; H, 3·0; N, 9·3; O, 31·95%), $\nu_{max.}$ (in paraffin) 1739, 1610, 1540, and 1350 cm.$^{-1}$, $\lambda_{max.}$ (in MeOH) 223, 252 (infl.), 443 and mμ (log ε 4·39, 4·02, and 2·35). Sublimation of the water-soluble products gave methyloxamide (1·8 mg.), m. p. 232—234°.

Oxidation of the Yellow Indole (V) with Manganese Dioxide.—(*a*) The indole (V) (65 mg.), freshly prepared manganese dioxide [10] (0·5 g.), and benzene (5 ml.) were heated under reflux for 1 hr. The mixture was filtered, the manganese dioxide was extracted with chloroform, and the combined filtrate and extract were evaporated. The residue was adsorbed from benzene on silica gel. *7-Chloro-1,2,4,10-tetrahydro-8,9-dimethoxy-3,10-dimethyl-1,2,4-trioxo-3H-pyrazino*-[1′,2′ : 1,5]*pyrrolo*[2,3-*b*]*indole* was eluted with benzene–ether (19 : 1) and separated as orange-red needles (22 mg.) (from chloroform–methanol), m. p. 312—313° (Found: C, 54·65; H, 4·05; Cl, 9·5; N, 10·2; OMe, 14·6; *N*-Me, 6·9; Me·NH_2 on hydrolysis,[3,4] 7·9. $C_{17}H_{14}ClN_3O_5$ requires C, 54·35; H, 3·75; Cl, 9·45; N, 11·2; 2OMe, 16·5; 2*N*-Me, 8·0; Me·NH_2, 8·0%), *m/e* 377, 375; 292, 290; 277, 275; and 234, 232, $\lambda_{max.}$ (in MeOH) 219, 257, 293, and 398 mμ (log ε 4·30, 4·45, 4·40, and 3·81), $\nu_{max.}$ (in $CHCl_3$) 1740, 1720, and 1675 cm.$^{-1}$.

(*b*) When the oxidation described above was repeated with manganese dioxide that was more than 3 weeks old, 5-chloro-6,7-dimethoxy-1-methylisatin [1] was obtained in 21% yield.

Action of Alkali on the Yellow Indole (V).—(*a*) The indole (V) (135 mg.), methanol (10 ml.), and 15% aqueous sodium hydroxide (1 ml.) were shaken together at 50° until homogeneous. The solution was acidified with acetic acid and heated at 90° until a precipitate was formed. This product, *2-methylcarbamoyl-5-chloro-6,7-dimethoxy-8-methylpyrrolo*[2,3-*b*]*indole*, was sublimed at 220°/0·1 mm. and recrystallised from chloroform–methanol (yield 31 mg.), then having m. p. 240—250° (decomp.) (Found: C, 56·05; H, 5·1; N, 12·9; O, 15·2; Me·NH_2 on hydrolysis,[3,4] 8·9. $C_{15}H_{16}ClN_3O_3$ requires C, 56·0; H, 5·0; N, 13·05; O, 14·9; Me·NH_2, 9·3%), *m/e* 323, 321; 292, 290; 277, 275; and 234, 232 (after deuteration,[7] 325, 323; 292, 290; 277, 275; and 234, 232),

[9] Morgan and Kingsbury, *Analyst*, 1959, **84**, 409.
[10] Mancera, Rosenkranz, and Sondheimer, *J.*, 1953, 2189.

$\lambda_{max.}$ (in MeOH) 235, 281, and 328 mμ (log ε 4·42, 4·39, and 4·44), $\nu_{max.}$ (in paraffin) 3440, 3210, 1640, 1615, and 1565 cm.$^{-1}$, τ (in pyridine) 5·90 (intensity 3), 5·95 (intensity 6), 6·73, and 6·90.

(*b*) Identical material (6 mg.) was obtained by similar treatment of the C_{17} trioxopiperazine (VI) (14 mg.).

Action of Alkali on the Dihydro-derivative (VIII).—The dihydro-derivative (73 mg.) in methanol (1 ml.) was treated with 15% sodium hydroxide solution (0·1 ml.). The solution was immediately diluted with water, acidified with acetic acid, and warmed to coagulate the precipitate, namely, N-(*5-chloro-6,7-dimethoxy-8-methylpyrrolo*[2,3-*b*]*indole-2-carbonyl*)-N-*methylalanine*, which separated from dioxan–ethanol as prisms (52 mg.), m. p. 220—222° (decomp.) (Found: C, 54·7; H, 5·3; N, 10·45; O, 20·3. $C_{18}H_{20}ClN_3O_5$ requires C, 54·9; H, 5·1; N, 10·65; O, 20·3%), $\lambda_{max.}$ (in MeOH) 236, 282, and 330 mμ (log ε 4·43, 4·31, and 4·37), $\nu_{max.}$ (in paraffin) 3240, 1705, 1540, and 1400 cm.$^{-1}$. Hydrolysis with 6N-hydrochloric acid gave *N*-methylalanine and no volatile base. When the indole (IX) (12 mg.) was heated with acetic anhydride and sodium acetate under reflux for 10 min. the dihydro-derivative (VIII) (6 mg.) was obtained.

Isolation of N-*methylalanine.*—(*a*) The dihydro-derivative (VIII) (0·35 g.) was sealed in a glass tube with concentrated hydrochloric acid (30 ml.). The solid slowly dissolved and a colourless precipitate separated. The tube was heated at 100° for 18 hr., then the mixture was diluted with water and extracted with ethyl acetate. The aqueous phase was evaporated and the residue (0·32 g.) dissolved in water (10 ml.). The resin, Amberlite IRA-400 (20 g.), was stirred with dilute 2N-sodium hydroxide (100 ml.) at 40—45° for ½ hr., then washed 3 times with water and made into a slurry with water in a column fitted at its lower end with a capillary (0·5 mm. bore, 7 cm. long). The column was washed with water until the effluent was neutral, then with 2N-hydrochloric acid (50 ml.), with water until the effluent was chloride-free, with 2N-sodium hydroxide (50 ml.) and finally with water until the effluent was neutral. The capacity of the column as prepared was equivalent to 40 ml. of N-alkali. The aqueous solution containing the amino-acid was run on to the column which was then washed with water (300 ml.) until the effluent was colourless. *N*-Methylalanine was displaced with N-acetic acid (150 ml.) which on evaporation gave 62 mg (65%) of this product which was dissolved in the minimum volume of hot water, treated with charcoal (0·1 g.), and after filtration treated with acetone until the solution was turbid. After 24 hr. at −10° the colourless crystals [38 mg., m. p. 278—280°, $\nu_{max.}$ (in KBr) 3570, 3010, 2440, 1610br, 1400, and 1360 cm.$^{-1}$], were collected; it gave a tosyl derivative, m. p. 106°, identical with an authentic specimen.[11]

(*b*) DL-*N*-Methyl-*N*-tosylalanine (2·5 g.) was suspended in liquid ammonia (300 ml.) and treated with sodium (*ca.* 4 g.) until a permanent blue colour was obtained. Amberlite resin IRC-50 (30 g.; H^+ form) was added during 1 hr. and then the ammonia was allowed to evaporate.[12] The residue was treated with water, the resin was filtered off and washed with water, and the combined aqueous solutions were extracted with ether. The aqueous phase was evaporated and the residue taken up in hot water (20 ml.). The cold solution was filtered and the product (0·8 g.; m. p. 260—275°) obtained after evaporation of the filtrate was recrystallised as described above. Paper chromatography showed it to be contaminated with alanine. A pure specimen of DL-*N*-methylalanine was therefore obtained by paper chromatography as described by Sheehan *et al.*; [13] it was identical with the amino-acid from the hydrolysis of the dihydro-derivative (VIII).

Hydrogenation of the C_9 *Enol Acetate* (II) *and Acid Hydrolysis of the Product.*—The C_9 acetate (11·8 mg.) was hydrogenated in methanol over 5% palladium–charcoal at room temperature and pressure until no more gas was absorbed (*ca.* 3 mol.). The product [10 mg.; $\nu_{max.}$ (film) 1650, 1450, 1315, and 1250 cm.$^{-1}$] was treated with concentrated hydrochloric acid (0·5 ml.) and water (0·3 ml.). After being heated at 120° (sealed tube) for 24 hr. the colourless solution was evaporated and the residue (11 mg.) dissolved in water (1 ml.). This solution (10 μl.) was chromatographed on Whatman No. 3MM paper with the acid and the basic solvent systems described previously.[3] The papers developed with the acid system were sprayed with the Folin reagent and two blue spots of the same R_F as serine and alanine were seen. The slower-moving spot was also detected by the periodate–benzidine reagent.[14] The fastest-moving spot on these papers

[11] Fischer and Bergmann, *Annalen*, 1913, **398**, 96.
[12] Ovchinnikov, Ivanoc, and Kiryushkin, *Izvest. Akad. Nauk S.S.S.R., Otdel. khim. Nauk*, 1962, 2046.
[13] Sheehan, Maeda, Sen, and Stock, *J. Amer. Chem. Soc.*, 1962, **84**, 1303.
[14] Cifonelli and Smith, *Analyt. Chem.*, 1955, **27**, 1501.

was red and had the same R_F value as *N*-methylalanine. Only 3 spots were observed on the chromatograms developed with the basic solvent system and they had identical R_F values (in increasing order) with serine, alanine, and *N*-methylalanine. The solution of amino-acids (20 μl.) was applied to Whatman No. 3MM paper and 6·6, 16·5, 33·1, 49·6, and 60·2 μg. of *N*-methylalanine were run on the same paper. The chromatogram was developed at 20° for 90 hr. with the basic solvent system,[3] was dried and sprayed with a solution (100 ml.) of ninhydrin (1 g.) in phosphate buffer (M/15; pH 7) saturated with n-butyl alcohol, and the spots were developed, eluted, and determined as described by Russell.[15] The analysis was similarly carried out for serine and alanine. The results indicated the presence of *N*-methylalanine (5·8 mg.), alanine (3·5 mg.), and serine (1·8 mg.) in the total hydrolysate. Serine in the hydrolysate was also estimated by the method of Frisell, Meech, and Mackenzie.[16] This result indicated 1·1 mg. of serine in the total hydrolysate.

Alkaline Hydrolysis of the C_9 *Enol Acetate.*—The enol acetate (II) (3 mg.) and N-sodium hydroxide (0·5 ml.) were heated at 100° in a sealed tube for 10 min. The mixture was evaporated, the residue dissolved in the minimum volume of water, and the presence of acetic acid shown by paper chromatography as previously described.[5]

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[15] Russell, *J. Chromatog.*, 1960, **4**, 251.
[16] Frisell, Meech, and Mackenzie, *J. Biol. Chem.*, 1954, **207**, 709.