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# STRUCTURE OF MONASCOFLAVIN[1]

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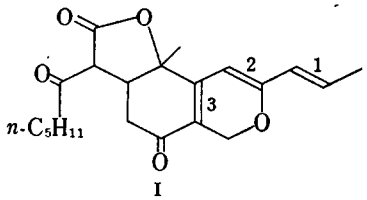
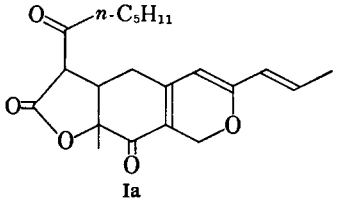
**Abstract**—Structure I (or Ia) is deduced for monascoflavin.

MONASCOFLAVIN[2,3] (Monascin[4,5]), $C_{21}H_{26}O_5$ is the yellow pigment produced by *Monancus purpureus* Wentii, and together with monascorubrin,[6] is the main constituent pigment of the Taiwan wine, Hong Ru. The name monascoflavin is preferred over monascin since the latter has already been used for designating the yellow pigment isolated from *M. paxii* Lingelsheim.[7]

Evidence leading to structure I (or Ia) will be described in this paper. The material was prepared by recrystallizing the crude monascoflavin collected by one of us (H. N.) some 25 years ago from ethanol or by treating the recrystallization mother liquid from monascorubrin[6] with hydrogen peroxide; the latter procedure destroyed selectively the remaining monascorubrin.




I




Ia

The IR and UV data of some pertinent derivatives are listed in Table 1. The dihydro-, tetrahydro- and hexahydro-derivatives are those in which the double bonds (1), (2) and (3) in I have been successively hydrogenated as evidenced by the shifts in UV maxima.

The presence of a n-$C_5H_{11}CO$ side-chain was shown by the production of n-hexanoic acid upon oxidation with potassium permanganate[4] or fusion with alkali,[3] and production of n-amylamine upon Beckmann rearrangement and hydrolysis of the

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[1] M. Ohashi, S. Yamamura, A. Terahara and K. Nakanishi, *Bull. Chem. Soc. Japan* **33**, 1630 (1960): short communication. Paper read at the 1st IUPAC Symposium on Natural Products, Australia, August, 1960.
[2] H. Nishikawa, *J. Agric. Chem. Soc. Japan* **2**, 688 (1926).
[3] H. Nishikawa, *J. Agric. Chem. Soc. Japan* **8**, 1007 (1932).
[4] H. Salmon and P. Karrer, *Helv. Chem. Acta* **15**, 18 (1931); P. Karrer and A. Geiger, *Ibid.* **25**, 289 (1941).
[5] A. D. G. Powell, A. Robertson and W. B. Whalley, *Chem. Soc. Special Publ.* No. 5, p. 27 (1956).
[6] This issue, p. 1171.
[7] Lingelsheim, *Hedwigia* **57**, 253 (1916).

oxime. Monascoflavin and hydrogenated derivatives reacted with ammonia and various amines to afford amides, which lacked the 1786 $cm^{-1}$ IR band and which had the 1720 $cm^{-1}$ band displaced considerably to lower frequencies; for example tetrahydromonascoflavin afforded the hexyl amide II (R = n-$C_6H_{13}$, Table 1) when reacted with n-hexylamine. However, the UV absorptions of the amides were similar to those of the parent compounds.

When solutions of monascoflavin or the hydromonascoflavins were made alkaline a 285–290 m$\mu$ peak either appeared or was intensified, whilst the maxima of other

TABLE 1. INFRARED AND ULTRAVIOLET ABSORPTIONS

| Compound | Solvent | IR | | | UV |
|---|---|---|---|---|---|
| | | —COO— | —CO— | $\Delta^{\alpha\beta}$CO | $\lambda_{max}^{MeOH}$ ($\log^{MeOH}$) |
| Monascoflavin (I) | $CHCl_3$ | 1786 | 1720 | 1673 | 225 (4·21), 288 (3·41)[a] 385 (4·21) |
| Dihydromonascoflavin | $CHCl_3$ | 1791 | 1723 | 1672 | 220 (3·77), 237 (3·61) 364 (3·91) |
| Tetrahydromonascoflavin | $CHCl_3$ | 1790 | 1725 | 1703 | 244 (4·03)[b] |
| Hexahydromonascoflavin | $CHCl_3$ | 1778 | 1723 1742[c] | | |
| Hexyl amide (II, R = $C_6H_{13}$)[d] | $CHCl_3$ | | 1702 | 1665 | 241[b] |
| Nortetrahydromonascoflavin (XIII) | $CCl_4$ | | 1712 | 1668 | 242[b] |
| Tetrabromomonascoflavin (XIV) | $CHCl_3$ | 1786 | 1711 | 1690 | 225 (3·93), 365 (3·95) |

[a] Enolate band.

[b] The difference with the calculated $\lambda_{max}$ of 249 m$\mu$ is presumably caused by a transannular effect of the oxygenic p-electrons on the $\alpha,\beta$-unsaturated system. A similar effect has been reported for nitrogen: A. Marchant and A. R. Pinder, *J. Chem. Soc.* 327 (1956).

[c] This unusually high $\nu_{C=O}$ value for a six-membered ring ketone is due to ring strain imposed by the $\gamma$-lactone. A similar effect is observed when the 1703 $cm^{-1}$ band in tetrahydromonascoflavin is compared with the 1668 $cm^{-1}$ band in nortetrahydromonascoflavin (XIII) in which the lactone is cleaved. The acetate of XIII also had its ring-ketone absorption at the normal position of 1675 $cm^{-1}$ ($CCl_4$).

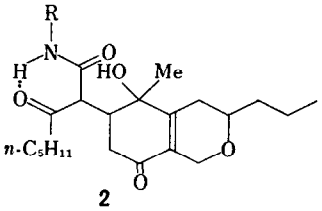
[d] Amide I and II bands at 1665 and 1520 $cm^{-1}$, respectively.

bands remained practically unaltered. Thus there exists two separate chromophores, one of which is a $\beta$-dicarbonyl unit that gives rise to the enolate UV absorption. Presence of a $\beta$-dicarbonyl unit is supported by the positive ferric chloride tests shown by monascoflavin and hydrogenated derivatives, and in conjunction with the spectroscopic behavior of the amides, this unit is shown to be a $\beta$-keto-lactone. The optical properties of the mono-2,4-dinitrophenylhydrazone (no 1720 $cm^{-1}$ band, $\lambda_{max}^{EtOH}$ 361 m$\mu$) and di-2,4-dinitrophenylhydrazone (no. 1720 and 1673 $cm^{-1}$ bands, $\lambda_{max}^{EtOH}$ 350 and 414 m$\mu$) indicated that an unsaturated ketone was present in addition to the saturated ketone of the $\beta$-keto-$\gamma$-lactone moiety. Although the UV maxima of 2,4-dinitrophenylhydrazones are known to undergo red shifts in basic media,[8] this was not the case

[8] C. J. Timmons, *J. Chem. Soc.* 2613 (1957).

with the 355 mμ maxima exhibited by the two hydrazones; only the 414 mμ peak was shifted to 496 mμ in base.

This observation also can be explained by formation of a hydrogen bond in the β-keto-γ-lactone derivatives (III).

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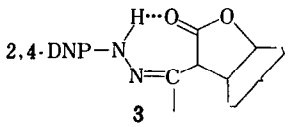


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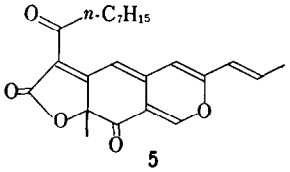


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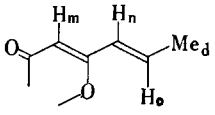


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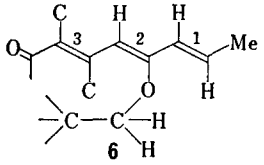


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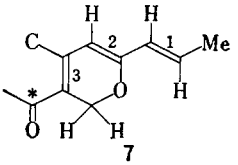


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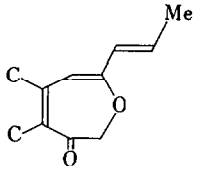


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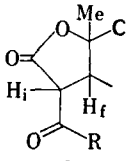


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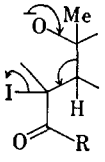



10

Ozonolyses of monascoflavin and dihydromonascoflavin gave acetaldehyde and butyric acid, respectively, while base degradation gave crotonic acid and butyric acid, respectively. These results show that the unit IV or its vinylog similar to that found in monascorubrin (V) should be present to permit the occurrence of a β-diketone cleavage This was supported by the $ABX_3$ type NMR signals of monascoflavin, o, n, and d (Fig. 1), which were absent in the dihydro-derivative. In tetrahydromonascoflavin the olefinic singlet $H_m$ had also disappeared, but the IR and UV data suggested the presence of still another double bond that was present as a tetrasubstituted α,β-unsaturated ketone; the low intensity of the 1630 $cm^{-1}$ C=C stretching absorption and the high intensity of the 244 mμ peak further showed it to be transoid rather than cisoid. Unlike monascorubrin, no formic acid is produced upon alkaline degradation of monascoflavin, and this coupled with the AB type quartet at 5·1, which is also present in the dihydro derivative but not in the tetrahydro derivative,[9] places a methylene group adjacent to the enol ether oxygen in IV. Thus VI is derived.

Furthermore, in order to account for the 364 mμ maximum of dihydromonascoflavin, double bonds 2 and 3 should constitute a homoannular diene (bathochromic

[9] M. Ohashi, A. Terahara, K. Nakanishi, I. Yamaguchi and N. Hayakawa, *Bull. Chem. Soc. Japan* 33, 1312 (1960).

The "hexahydro" and "octahydro" monascoflavin in the mentioned paper should be corrected to the "tetrahydro" and "hexahydro" derivatives, respectively.

shift of 39 mμ[10]), and this leads to the expression VII having a calculated UV maximum of 352 mμ.[11]

The particular allylic position of the methylene also accounts for the appearance of its NMR signal at the rather low field of τ 5·1 for cyclic —$CH_2O$— groups (usually τ 6·4).[12]

Partial structure VIII for which the UV maximum cannot be calculated empirically, was discarded because the structure of monascoflavin derived thereof could not be rationalized easily with biogenetic results,[13] which were in agreement with the conventional acetate theory.

Apparently the new 1742 $cm^{-1}$ peak in the IR spectrum of hexahydromonascoflavin (Table 1) is associated with the unsaturated carbonyl (asterisked) in VII, which becomes saturated in the hexahydro compound. However, it is not this ketone that constitutes the n-$C_5H_{11}CO$ side-chain mentioned above, for there is no reason that it should absorb at this unusually high frequency for a saturated aliphatic ketone. Accordingly, it is the remaining saturated ketone, namely that of the β-keto-γ-lactone group, that carries the n-pentyl group. The enhanced frequency of the asterisked carbonyl in VII is also encountered in tetrahydromonascoflavin, which absorbed very high for an α,β-unsaturated ketone (1703 $cm^{-1}$). As described in footnote *c* (Table 1), the effect is attributed to ring strain rather than electronic effects of other oxygen functions.

The conspicuous NMR singlet at 8·54 would suggest a methyl group attached to a double bond, and indeed the grouping =$C(CH_3)$—CH= was suggested by Karrier and Geiger[4] on the basis that ozonolysis of monascoflavin affords methylglyoxal in addition to acetaldehyde. However, the singlet persists in di-, tetra-, and hexahydromonascoflavin, and furthermore, methylglyoxal results from ozonolysis of substances such as 2,6-dimethyl-γ-pyrone[14] and monascorubrin.[6]

It follows that the methyl group giving rise to the low-field signal can only be attached to the lactone ring as shown in IX; this also explains the positive iodoform reactions of monascoflavin and all hydrogenated derivatives, for hydrolysis would lead to a γ-ketol capable of giving iodoform reactions according to a mechanism shown in X.[15]

The i-doublet at τ 6·25 is assigned to $H_i$ (IX), which is coupled to $H_f$ ($J_{fi}$ 13 cps).

Only three C—$CH_3$ groups are present and hence the combination of units VI and VIII to give monascoflavin is limited to the four possibilities, I, Ia, XI or XII. In formulae XI and XII, hydrolysis of the lactone ring leads to a β-ketol, which would be expected to undergo facile dehydration and aromatization or retro-aldol cleavage. On the contrary, stable amides of the general type II are formed by reacting with amines; also, boiling of tetrahydromonascoflavin with 20% alkali under nitrogen for several hours yielded the decarboxylated nor-compound XIII, which still retained the hydroxyl group as shown by the 3400 $cm^{-1}$ O—H stretching absorption. Thus the

[10] L. F. Fieser and M. Fieser, *Steroids* p. 19. Reinhold, New York (1959).

[11] Unpublished empirical calculations by H. Nakata have shown that +28 mμ, rather than +18 mμ[10], should be considered for each β- or γ-alkoxyl substituent. The other values employed were those given by Fieser and Fieser.

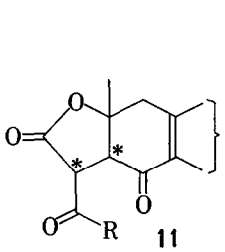
[12] L. M. Jackman, *Application of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry* p. 55. Pergamon Press, London (1959).

[13] To be published.

[14] P. S. Bailey, *Chem. Rev.* **58**, 925 (1958).

[15] D. H. R. Barton, S. K. Pradhan, S. Sternhell and J. F. Templeton, *J. Chem. Soc.* 255 (1961).

hydroxyl group is stable to alkali. In addition, XI and XII are also incompatible with biogenetic studies,[13] which showed that monascoflavin is composed of two polyketomethylene chains and that the angular methyl originates from an extra one-carbon source. In XI and XII, the two polyketomethylene chains should have to be connected through the asterisked carbon atoms, but both of these originate from methylene groups and are unsuited for aldol condensation. The acetate theory requires extra methyl groups to be attached to the methylene group[16] but again this is not what is found in the two structures.



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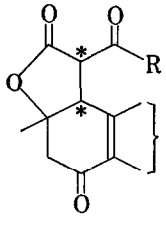

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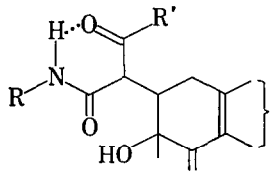

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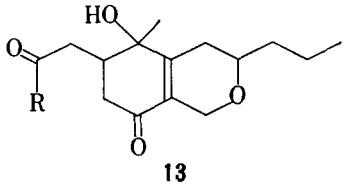

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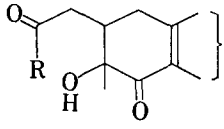
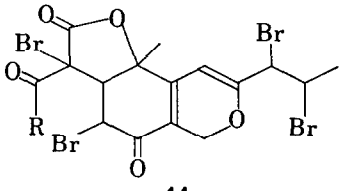


13a



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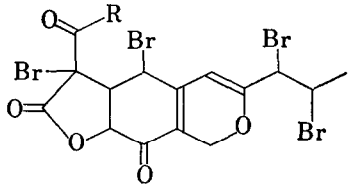


14a

Of the remaining alternatives, I or Ia, the former appears to be favored on grounds of the following observations. Treatment of monascoflavin with bromine afforded tetrabromomonascoflavin having an UV spectrum quite similar to that of dihydromonascoflavin (Table 1). It gives no coloration with ferric chloride and the spectrum is not changed upon addition of base. Accordingly, it can be represented either as XIV (from I) or XIVa (from Ia). The $+17$ cm$^{-1}$ shift of the monascoflavin 1673 cm$^{-1}$ band can be reconciled with structure XIV by an equatorial $\alpha$-bromine but not with XIVa. In addition, tetrahydromonascoflavin amide was found to consume neither periodic acid nor lead tetraacetate; although the evidence is not decisive,[17] it could be rationalized more easily by II (R = H) than IIa (R = H), which is an $\alpha$-ketol. On the other hand, Ia is more closely related to the structure of the copigment monascorubrin V.[6]

Robertson *et al.*[18] are in favor of structure Ia on the basis of the observation that

[16] A. J. Birch, P. Fitton, E. Pride, A. J. Ryan, H. Smith and W. B. Whalley, *J. Chem. Soc.* 4576 (1959).

[17] E. Baer, *J. Amer. Chem. Soc.* **62**, 1597 (1940); **64**, 1416 (1942).

[18] The authors are grateful to Professor W. B. Whalley for informing us of their results at the 1st IUPAC Symposium on Natural Products, Australia, August 1960. The results have been published recently after submission of this paper: B. C. Fielding, J. S. E. Holker, D. F. Jones, A. D. G. Powell, K. W. Richmond, Alexander Robertson and W. B. Whalley, *J. Chem. Soc.* 4579 (1961).

nortetrahydromonascoflavin gave a positive iodoform test when reacted with periodic acid and then with hypoiodite. However, nortetrahydromonascoflavin itself, like monascoflavin and all hydrogenated derivatives, was found to give a positive iodoform test owing to its $\gamma$-ketol moiety[15] X, and thus the evidence is not conclusive.

The following analysis of the NMR spectrum of monascoflavin (Table 2) is applicable to both alternative structures, but is based on I in view of the slight preference for it deduced from the above-mentioned IR spectrum of the tetrabromo-derivative. The chemical shifts and coupling constants were calculated by the usual method.

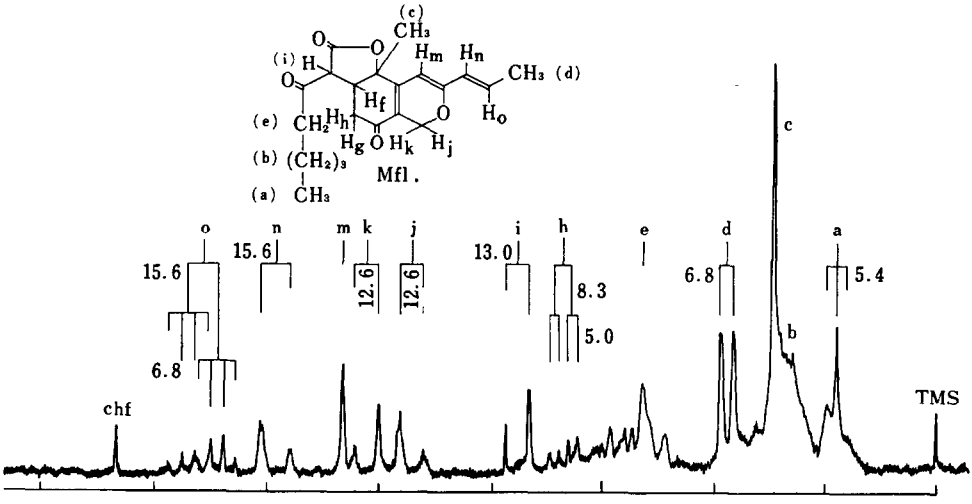



FIG. 1. NMR spectrum of monascoflavin at 60 Mc in $CDCl_3$ (in $\tau$)

TABLE 2. NUCLEAR MAGNETIC RESONANCE SPECTRUM OF MONASCOFLAVIN

| Signal | $\tau$ value in ppm* | J in cps | Remarks |
|---|---|---|---|
| c | 8·54, s | | Shifted lower by 0·2 ppm in pyridine |
| d | 8·12, d | $J_{do} = 6·8$ | Shifted to normal methyl region in dihydro-deriv |
| e | 7·4, m | | |
| h | 6·7, q | $J_{th} = 5·0$<br>$J_{gh} = 8·3$ | Corresponds to X component of ABX system; the AB components, $H_g$ and $H_f$ (further coupled to $H_i$), overlap with $H_e$ proton signals |
| i | 6·25, d | $J_{if} = 13·0$ | Shifted to 5·5 in pyridine, which indicates that it is associated with a proton near oxygenic functions[20] |
| j<br>k | 5·24<br>4·94 , q | $J_{jk} = 12·6$ | Still present in dihydro-deriv. Nearby ring ketone also contributes to lowering of field |
| m | 4·68, s | | Present in dihydro-, but absent in tetrahydro-deriv |
| n<br>o | 4·04, d<br>3·42 | $J_{no} = 15·6$<br>$J_{do} = 6·8$ | AB component of $ABX_3$ system. J values suggest propenyl chain is *trans*. Disappears in dihydro deriv |

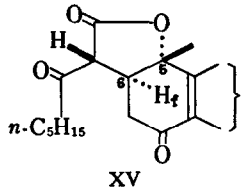
* s: singlet; d: doublet; q: quartet; m: multiplet.

[19] Ref. 12, p. 89.

[20] G. Slomp and F. MacKellar, *J. Amer. Chem. Soc.* **82**, 999 (1960).

If its stereochemistry is considered, monascoflavin could be represented either as XV or its mirror image. Namely, because the hydroxyl group in the amides II and the nortetrahydro-derivative XIII cannot be dehydrated, the $C_5$—O and $C_6$—$H_f$ bonds are cis oriented, and thus the lactone ring is transfused. Further, the two hydrogens $H_f$ and $H_i$ are probably *anti–trans* in view of the large coupling constant J.[21,22]

The same deductions will apply to Ia as well. Although monascoflavin exhibited a plain positive rotatory dispersion curve, and tetrahydro- and hexahydro-monascoflavin exhibited positive cotton effect curves, it is still premature to draw conclusions on the absolute configuration.




XV

## EXPERIMENTAL

M.ps. were determined on a micro hot-stage and are uncorrected. The UV spectra were measured with Beckmann DK-2 and Hitachi-EP 2 recording spectrophotometers, the IR spectra with a Nihon Bunko 301 spectrophotometer equipped with rocksalt prisms and the NMR spectra with Varian A-60 and V-4300 models.

*Monascoflavin* (I). The material collected by one of us (H. N.) 25 years ago from *Monascus purpureus* Wentii and stored in a can was recrystallized several times from ethanol. Another source was the mother liquid from the recrystallization of crude monascorubrin;[6] the ethanol solution was concentrated *in vacuo* and the dark brown residue was treated overnight with 3% ethanolic hydrogen peroxide in the ice-box, upon which the monascorubrin was destroyed. The precipitates of monascoflavin were collected by filtration and recrystallized from ethanol, plates, m.p. 143–145° (Found: C 70·44, 70·35; H, 7·43, 7.51. $C_{21}H_{26}O_5$ requires: C, 70·37; H, 7·31%; C—$CH_3$: 2·44 and 2·51 moles). $[\alpha]_{700}^{16}$ +418° (0·0062 in ethanol); plain positive RD curve. UV: $\lambda_{max}$(MeOH) 225, 228 and 385 mμ (log ε 4·21, 3·41 and 4·21). IR (KBr): 1788, 1720, 1670, 1600 and 1522 $cm^{-1}$. It gave a positive iodoform test.

*Dihydromonascoflavin.* A suspension of monascoflavin (I, 1 g) in 50 ml ethanol was hydrogenated with 0·1 g 5% palladium-barium sulfate for 30 min, when 60 ml hydrogen was absorbed. After removal of the catalyst and concentration of the solution *in vacuo* 0·82 g of yellow crystals, m.p. 122° (ethanol) were obtained. (Found: C, 70·0, 70·0; H, 8·18, 7·77. $C_{21}H_{28}O_5$ requires: C, 70·0; H, 7·83%). UV: $\lambda_{max}$(MeOH) 220, 237 and 364 mμ (log ε 3·77, 3·61 and 3·91). IR (KBr): 1780, 1724, 1668, 1631 and 1551 $cm^{-1}$. It gave a pale pink color with an ethanolic solution of ferric chloride.

*Tetrahydromonascoflavin.* A suspension of monascoflavin (I, 1·22 g) in 50 ml ethanol was hydrogenated with 0·5 g 5% palladium–barium sulfate at 40°. When 215 ml hydrogen had been absorbed (about 5 hr), the catalyst was removed and the solvent was concentrated *in vacuo* to give colorless needles, 0·9 g, m.p. 136–137° (ethanol) (Found: C, 70·0, H; 8·25. $C_{21}H_{30}O_5$ requires: C, 69·6; H, 8·34%). UV: $\lambda_{max}$(MeOH) 244 μm (log ε 4·03), $\lambda_{max}$ (0·1 N NaOH–EtOH) 246 and 286 mμ (log ε 3·92 and 4·19). IR (KBr): 1785, 1722, 1689 and 1635 $cm^{-1}$. ORD (0·1 in methanol), 14·5°: $[\alpha]_{500}$ +340°, $[\alpha]_{400}$ +780°, $[\alpha]_{350}$ +1340°, $[\alpha]_{320}$ +990°, $[\alpha]_{310}$ +1130°.

*Hexahydromonascoflavin.* An ethanolic suspension of 1 g of monascoflavin (I) was hydrogenated with 1·05 g 5% palladized charcoal. When hydrogen uptake (230 ml) had ceased, the catalyst was removed and the solvent was evaporated *in vacuo* to give colorless needles (0·5 g), m.p. 211–212° (ethanol) (Found: C, 69·1; H, 8·75. $C_{21}H_{32}O_5$ requires: C, 69·2; H, 8·85%). IR (KBr): 1776 and 1707 $cm^{-1}$. ORD (0·098 in methanol), 15·5°: $[\alpha]_{500}$ +25·5°, $[\alpha]_{350}$ +120°, $[\alpha]_{330}$ +210°, $[\alpha]_{305}$ −97°.

[21] M. Karplus, *J. Chem. Phys.* **30**, 11 (1959).

[22] H. Conroy in *Advances in Organic Chemistry* Vol. II; p. 311. Interscience, New York (1960).

*Oxidation of monascoflavin* (I) *with potassium permanganate.* Powdered potassium permanganate (3·45 g) was added gradually with shaking to a suspension of monascoflavin (I, 0·5 g) in 40 ml hot water until the color was no longer discharged. The manganese dioxide was filtered, washed with hot water and the combined filtrate was extracted with ether. From the ether layer, acetic acid and hexanoic acid were identified by paper-chromatography in the solvent system of butanol saturated with 1·5 N $NH_4OH$. $R_f$ values: 0·11 (acetic acid), 0·29, 0·46 and 0·60 (hexanoic acid).

*Oxidation of tetrahydromonascoflavin with potassium permanganate.* The oxidation of tetrahydromonascoflavin similarly yielded hexanoic acid ($R_f$, 0·61) and butyric acid ($R_f$, 0·35).

*Nortetrahydromonascoflavin* (XIII, R = n-$C_5H_{11}$). A suspension of tetrahydromonascoflavin (0·3 g) in 20 ml 20% NaOH aq was refluxed for 2·5 hr in a current of nitrogen. After acidification with hydrochloric acid, the solution was extracted with ether. The ether layer was washed with dil hydrochloric acid, evaporated *in vacuo* and the residual syrup was purified by chromatography on acid-washed alumina. Elution with benzene–ether (1:1) gave a colorless solid m.p. 41–44°. UV: $\lambda_{max}$(MeOH) 242 m$\mu$ IR: 3400, 1712, 1668 and 1648 $cm^{-1}$. The solid was refluxed with acetic anhydride and pyridine for 1 hr and purified by chromatography on acid-washed alumina; a colorless oil having IR absorptions (in $CCl_4$) at 1740 (acetate), 1714 (ketone), 1675 and 1652 $cm^{-1}$ ($\alpha,\beta$-unsaturated ketone) was obtained.

*Beckmann rearrangement of monascoflavin oxime.* A solution of monascoflavin (I, 0·2 g) and 0·1 g hydroxylamine hydrochloride in 5 ml ethanol was refluxed for 1 hr. The solution was poured into water, and the precipitates were dissolved in 1 g of polyphosphoric acid and heated at 150° for 15 min. After cooling, the solution was poured into water and extracted with ether. Recovery of the product gave a brown solid, which was suspended in 50% sulfuric acid and refluxed for 30 min. After cooling and addition of sodium hydroxide, the aqueous solution was steam-distilled and the distillate was extracted with ether. A drop of dil hydrochloric acid was added and the ether layer was evaporated *in vacuo*. Amylamine was identified by paper-chromatography (Toyo filter paper No. 51, butanol–acetic acid–water (4:1:5), $R_f$ value 0·67).

*Degradation of monascoflavin* (I) *with alkali.* Monascoflavin (I, 0·5 g) was heated with 50 ml 2 N KOH aq under distilling conditions in a current of nitrogen for 1 hr. Sufficient distilled water was added to maintain the volume. The distillate was passed through 2,4-dinitrophenylhydrazine-2 N HCl when a few mg of the acetaldehyde derivative was obtained. After acidification, the residue was steam-distilled. The distillate gave negative chromotropic acid and mercuric chloride tests for formic acid. The distillate was extracted with ether, and the ether layer was concentrated *in vacuo* after addition of a drop of ammonia. Crotonic acid and acetic acid were detected from the residual syrup by paper-chromatography with Toyo filter paper No. 51, using butanol saturated with 1·5 N $NH_4OH$ as solvent; $R_f$ values 0·27 and 0·11, respectively.

*Monascoflavin amide.* Aqueous ammonia (30 ml) was added to a solution of monascoflavin (I, 1 g) in 10 ml ethanol and allowed to stand for 2 days, when the amide slowly separated. Re-precipitation from ethanol–water (1:1) gave a yellow powder (0·5 g), m.p. 152–153° (Found: C, 66·5; H, 7·88; N, 3·76. $C_{21}H_{29}O_5N$ requires: C, 67·2; H, 7·79; N, 3·73%). UV: $\lambda_{max}$(MeOH) 226 and 382 m$\mu$. IR (KBr): 3373, 3193, 1713, 1645 and 1536 $cm^{-1}$.

*Dihydromonascoflavin amide.* A suspension of dihydromonascoflavin (0·1 g) in 3 ml aqueous ammonia containing a few drops of ethanol was allowed to stand overnight. The precipitates (0·08 g) were crystallized from ethanol, m.p. 188–189° (Found: C, 67·4, 67·1; H, 8·53, 8·55; N, 4·01. $C_{21}H_{31}O_5N$ requires: C, 66·8; H, 8·28; N, 3·71%). UV: $\lambda_{max}$ (MeOH) 220, 235 and 356 m$\mu$ (log $\varepsilon$ 3·80, 3·53 and 3·94). IR (KBr): 1722, 1668, 1642, 1598 and 1551 $cm^{-1}$.

*Tetrahydromonascoflavin amide.* A suspension of tetrahydromonascoflavin (0·1 g) in 30 ml aqueous ammonia containing a few drops of ethanol was allowed to stand overnight. The precipitates were crystallized from ethanol giving colorless needles (0·06 g), m.p. 186–187° (Found: C, 66·4; H, 8·53; N, 3·60. $C_{21}H_{35}O_5N$ requires: C, 66·1; H, 9·25; N, 3·52%). UV: $\lambda_{max}$ (MeOH) 241 m$\mu$ (log $\varepsilon$ 4·00). IR (KBr): 1720, 1666, 1645 and 1600 $cm^{-1}$.

*Tetrahydromonascoflavin hexylamide* (II). Tetrahydromonascoflavin (0·1 g) was added to 3 ml hexylamine, the mixture was left overnight, and the colored solution was poured into a large amount of water. The precipitates were purified by alumina chromatography and elution with benzene containing a few drops of ethanol, needles (0·08 g), m.p. 177–178° (ethanol) (Found: C, 70·2; H, 9·74; N, 2·93. $C_{21}H_{43}O_5N$ requires: C, 70·3; H, 9·39; N, 3·03). I R (KBr): 1713, 1670, 1645 and 1530 $cm^{-1}$.

*Monascoflavin di-2,4-dinitrophenylhydrazone.* To an ethanolic solution (15 ml) of 2,4-dinitrophenylhydrazine (1 g) and 2 ml conc sulfuric acid, there was added 0·46 g monascoflavin (I), and the solution was allowed to stand overnight to yield red purple precipitates (0·27 g), which were reprecipitated from ethanol. UV: $\lambda_{max}$ (EtOH) 350 and 414 m$\mu$ (log $\varepsilon$ 4·42 and 4·32), $\lambda_{max}$ (0·1 N NaOH–EtOH) 360 and 496 m$\mu$ (log $\varepsilon$ 4·36 and 4·45). IR (KBr): 1791, 1616, 1591, 1513 and 1501 cm$^{-1}$.

*Monascoflavin mono-2,4-dinitrophenylhydrazone.* A solution of 2 g 2,4-dinitrophenylhydrazine in 4 ml conc sulfuric acid was added to 0·36 g monascoflavin (I) dissolved in 20 ml of ethanol. Addition of water after 10 min gave red precipitates (0·32 g) which were precipitated from ethanol. UV: $\lambda_{max}$ (MeOH) 361 m$\mu$, $\lambda_{max}$ (0·1 N NaOH–MeOH) 371 m$\mu$. IR (KBr): 1781, 1668, 1618, 1595 and 1508 cm$^{-1}$.

*Ozonolysis of monascoflavin* (I). Ozonized oxygen (2–3%) was passed through a solution of monascoflavin (I, 0·28 g) in 20 ml of chloroform for 4 hr at 0°. The solvent was removed *in vacuo*, 50 ml water added, and after left standing overnight the solution was distilled into a saturated solution of 2,4-dinitrophenylhydrazine in 2 N HCl. Two hydrazones separated, one was crystallized from ethanol giving acetaldehyde 2,4-dinitrophenylhydrazone (0·05 g), m.p. 160°, identified by mixed m.p. and IR spectra, while the other ethanol-insoluble hydrazone was crystallized from nitrobenzene giving methylglyoxal bis-2,4-dinitrophenylhydrazone, m.p. 312–314°, identified by the above described methods with an authentic specimen.

*Ozonolysis of dihydromonascoflavin.* Ozonolysis of dihydronomascoflavin (0·25 g) for 1·5 hr at 0° and decomposition with water gave methylglyoxal and acetaldehyde (a few mg) identified as their 2,4-dinitrophenylhydrazones with authentic samples. In another run, the ozonide from 0·3 g of dihydromonascoflavin was steam-distilled after decomposition with water. The distillate was extracted with ether, the ether layer was dried and concentrated, and the residue was subjected to paper-chromatography on Toyo filter paper No. 51, using butanol saturated with 1·5 N $NH_4OH$ as solvent. The $R_f$ values, 0·61 and 0·35, were identical with those of hexanoic acid and butyric acid, respectively.

*Tetrabromomonascoflavin* (XIV). A solution of bromine in acetic acid was added to a solution of 1 g of monascoflavin (I) in 10 ml of the same solvent until the red color was no longer discharged. The solution was poured into ice water and the yellow precipitates were crystallized from ethanol to afford 0·9 g needles, m.p. 162–164° (Found: C, 36·8; H, 36·8. $C_{21}H_{24}O_5Br_4$ requires: C, 37·2; H, 3·58%). UV: $\lambda_{max}$ (MeOH) 225 and 365 m$\mu$ (log $\varepsilon$ 3·93 and 3·95). IR (KBr): 1785, 1716, 1691, 1610 and 1546 cm$^{-1}$. No color appeared when treated with alcoholic ferric chloride.

*Ozonolysis of tetrabromomonascoflavin* (XIV). Ozonized oxygen (2–3%) was passed through a solution of tetrabromomonascoflavin (XIV, 0·42 g) in 20 ml chloroform at 0° for 2 hr. Removal of the solvent *in vacuo*, decomposition of the residual ozonide with 50 ml water, and steam distillation into 2,4-dinitrophenylhydrazine in 2 N HCl gave a trace of acetaldehyde 2,4-dinitrophenylhydrazone and a few mg of methylglyoxal bis-2,4-dinitrophenylhydrazone, as identified by mixed m.p. and IR spectra.

*Oxidation of tetrahydromonoscaflavin and tetrahydromonascoflavin amide with lead tetraacetate.* Tetrahydromonascoflavin and tetrahydromonascoflavin amide were oxidized with lead tetraacetate in acetic acid at 30°. The consumption of the reagent was as follows:

| Time (hr) | 0·6 | 1·5 | 4·5 | 9·0 | 24·0 |
|---|---|---|---|---|---|
| Tetrahydromonascoflavin (moles) | 0·236 | 0·341 | 0·734 | 1·140 | 2·10 |
| Tetrahydromonascoflavin amide | 0·0 | 0·0 | 0·0 | 0·0 | 0·1 |

Standard techniques were employed for these analyses.

*Periodate oxidation of tetrahydromonascoflavin amide.* Standard techniques at room temp were employed for the analyses. The consumption of the reagent was as follows:

| Time (hr) | 0·5 | 1·0 | 2·0 | 5·0 |
|---|---|---|---|---|
| Tetrahydromonascoflavin amide (moles) | 0·0 | 0·0 | 0·0 | 0·0 |

The reaction was carried out on 0·0174 g of sample, and after the last measuring unchanged material was recovered.

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