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THE CHEMISTRY AND ^{13}C NMR ASSIGNMENTS OF OXALINE, A NOVEL ALKALOID FROM *PENICILLIUM OXALICUM*

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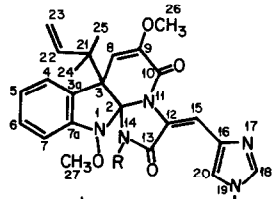
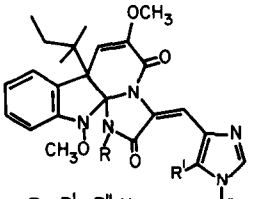
Abstract—Oxaline is the main alkaloid of several strains of *Penicillium oxalicum*. Structural studies on oxaline (**1a**) and the assignment of its ^{13}C NMR spectrum are described.

In the course of our continuing studies on mycotoxins, a toxigenic strain of *Penicillium oxalicum*, viz. M-555, was cultivated on a large scale on sterilized maize meal. Prolonged extraction of the mouldy material with chloroform–methanol gave secalonic acid D as the main toxic component[1] and oxaline (**1a**), a new alkaloid. Five different strains of *P. oxalicum* were subsequently grown in surface culture in a modified Czapek medium which contained 3% corn steep liquor. It is of chemotaxonomic importance that each of the five strains produced both secalonic acid D and oxaline. The chemical derivatization and degradation of oxaline together with application of physico-chemical techniques provided knowledge on the different fragments which constituted oxaline. The final construction of the oxaline molecule† was done by X-ray crystallography on a single crystal.[2]

The molecular formula of oxaline $C_{24}H_{25}N_5O_4$, indicated it to be highly unsaturated. Analysis and spectral data showed the presence of two methoxyl groups and of two exchangeable groups (N–H). It crystallized from nitromethane as orthorombic crystals, m.p. 220–221° and its long wavelength UV absorption λ_{max} 228 and 347 nm (log ϵ 4.32 and 4.39, respectively) is associated with the extended imidazole chromophore. The IR spectrum exhibited absorption at 3425 [N(14)–H], 3182 (N–H, imidazole), 2985, 2942, 1710, 1705, 1686 and 1640 cm^{-1}. The mass spectrum was uninformative, the main fragmentation was accounted by the loss of C_5H_8 (isoprenoid side-chain) from the molecular ion. This loss is dominant in all the derivatives of oxaline.

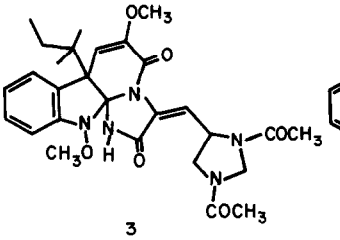


1a: R = R' = H
1b: R = R' = CH_3
1c: R = H, R' = $COCH_3$
1d: R = CH_3, R' = H



2a: R = R' = R'' = H
2b: R = R'' = H, R' = Br
2c: R = CH_3, R' = R'' = H
2d: R = R' = H, R'' = $COCH_3$

†A re-investigation of the X-ray data indicated that the imidazole N–H is not localized at one nitrogen atom only. However, only one form is shown on the drawings of the molecular formulae.



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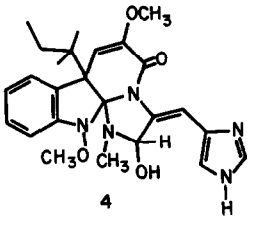

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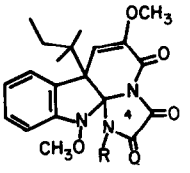
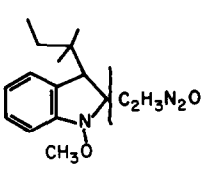


5a: R = H
5b: R = CH_3



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The 100 MHz ^1H NMR spectrum of oxaline had the following characteristics. An ABCD system due to the aromatic resonances (δ7.60, 4-H; δ7.09, 5-H; δ7.23, 6-H and δ6.92, 7-H) with the typical multiplets due to *ortho*- and *meta*-couplings. The specific assignments were calculated from the residual splittings in the off-resonance ^{13}C NMR spectrum of oxaline.[3] An ABX system was assigned to the protons which comprised the exocyclic double bond: δ_X6.10(22-H), δ_A4.95 and δ_B5.05(23-H_2) with S_{AX} = 18.0 Hz and S_{BX} = 10.0 Hz. Ten singlets at: δ12.72(1H), 9.70(1H), D_2O removed this and the previous N–H signal, 8.33(15-H), 7.44(18-H), 7.02(20-H), 5.14(8-H), 3.72 and 3.64(2 × OMe) 1.32 and 1.28 (*gem*-dimethyl group). The presence of the two exchangeable protons in oxaline was substantiated by permethylation to yield N,N'-dimethyloxaline (**1b**). Its IR spectrum lacked NH-absorption, while in the ^1H NMR spectrum two three-proton singlets at δ2.34 and δ3.54 were assigned to the N(14)-Me and N(19)-Me, respectively; the resonance at δ3.54 is characteristic for an N-methylimidazole. Two singlets at δ7.44 and δ8.36 are due to the protons belonging to the imidazole moiety in this compound.

Oxaline exhibits several unusual chemical characteristics. The steric congestion in the environment of the exocyclic double bond is exemplified by its unreactivity towards *m*-chloroperbenzoic acid and its resistance towards hydrogenation in ethanol over PtO_2. However,

hydrogenation in acetic acid over PtO_2 gave dihydro-oxaline, $C_{24}H_{27}N_5O_4$(2a). The ^{1}H NMR spectrum of this compound lacked absorption due to the exocyclic double bond while protons belonging to the ethyl group appeared as a complex multiplet at δ1.60(22-H_2) and a broadened triplet δ0.88(23-H_3). Kuhn–Roth oxidation of (2a) with concomitant distillation of volatile acids gave no 2,2-dimethylbutyric acid. The spectral data and the foregoing evidence established the presence of an isoprenoid unit which was linked in the reverse fashion, however, not to an sp^2 hybridized carbon atom as in other fungal dioxopiperazines.[4,5]

Treatment of oxaline with acetic anhydride:pyridine (1:1) readily furnished the labile N(19)-acetyloxaline, $C_{26}H_{27}N_5O_5$(**1c**), ν_{max} 3425 cm^{-1}[N(14)-H]. In its ^{1}H NMR spectrum the three-proton singlet (δ2.59) was assigned to the acetyl protons. The protons belonging to the imidazole unit shifted down-field to δ8.10(20-H) and δ8.65(18-H) and appeared as broadened one-proton singlets; the 15-H remained unchanged (δ8.36). The hydrogenation of the imidazole moiety is dependent upon the localization of the double bonds in the nucleus, by N-acetylation as the first step in the reduction sequence. Therefore, hydrogenation of oxaline in acetic acid even over prolonged periods gave only dihydro-oxaline (**2a**) (see before). Acetylation of dihydro-oxaline gave the N(19)-acetyl compound (**2d**). Hydrogenation of oxaline in acetic acid: acetic anhydride (1:1) over PtO_2 gave hexahydro-N,N′-diacetyloxaline, $C_{28}H_{35}N_5O_6$(**3**) as the major product and octahydro-N,N′-diacetyloxaline, $C_{28}H_{37}N_5O_6$, as the minor product. The UV spectrum of the hexahydro-derivative, λ_{max} 238 and 288 nm (log ϵ 4.24 and 4.06, respectively) lacked the long wavelength absorption associated with the extended imidazole chromophore. The ^{1}H NMR spectrum supported the structural assignments. The four contiguous aromatic protons resonated between δ6.9 and 7.6, with the signal of the 15-H (δ7.16, J = 10 Hz) superimposed upon this pattern. The protons of the imidazolidine moiety appeared as a quartet centred at δ5.03 (18-H_2) S = 10.0 Hz, a multiplet at δ3.80 (20-H_2) and a complex one-proton multiplet at δ6.14 (16-H). Irradiation at δ6.14 led to a collapse of the doublet at δ7.16(15-H) to a singlet and considerably simplified the multiplet at δ3.80(20-H_2). Two three-proton singlets at δ1.97 and δ2.10 were assigned to the N-acetyl protons. The proton at 8-H(δ5.16) was virtually unaffected. Octahydro-N,N′-diacetyloxaline was not characterized.

The bromination of dihydro-oxaline with pyridinium hydrobromide perbromide gave 20-bromodihydro-oxaline $C_{24}H_{26}BrN_5O_4$(**2b**). The ^{1}H NMR spectrum of this compound is practically identical to that of **2a** except for the lack of the 20-H resonance. The UV spectrum showed a slight bathochromic shift (3.5 nm) due to the bromination of the imidazole moiety. The location of the bromine atom at C-20 is in accordance with the relative rates of bromination[6] at different locations on the imidazole ring. All of the foregoing data provided evidence for the presence of the imidazole moiety in oxaline.

Oxaline is extremely labile towards dilute mineral acid. Upon treatment with dilute HCl the main product is formed through the formal addition of HCl to oxaline and the loss of the isoprene side-chain (C_5H_8) and methanol to give a red water-soluble compound, $C_{18}H_{14}N_5O_3Cl$ (*m/e* 383.0754). The compound was not characterized.

Oxaline is stable towards drastic treatment with aqueous alkali. The fusion of oxaline with powdered alkali at 180° for 3 min gave without acidification a number of reaction products in less than one per cent yield. Two of the compounds were analyzed by high resolution mass spectroscopy and had molecular formula $C_{13}H_{15}NO$ (*m/e* 201.114) and $C_{13}H_{16}N_2O$ (*m/e* 216.1269). Both compounds contained the aromatic nucleus and the isoprenoid side-chain. Oxaline is completely stable towards drastic reduction with LAH in dioxane or THF, presumably due to lactim formation in the 5-membered lactam. The N(14)-H in oxaline was protected by treatment with diazomethane to give in high yield the N(14)-methyloxaline, $C_{25}H_{27}N_5O_4$(**1d**) with ν_{max} 3190 cm^{-1} (N–H, imidazole) and δ[N(14)-Me] 2.48. The methylation of a lactam nitrogen by diazomethane is not uncommon; pyrimidine-4-one, quinazoline-2,4-dione and pteridin-2,4-dione react very readily with diazomethane to form the corresponding N–Me compounds.

N(14)-methyloxaline was hydrogenated and the N(14)-methyldihydro-oxaline smoothly reduced at room temperature by treatment with LAH in THF which led to reduction of the 13-CO group. Acetylation gave an unstable diacetyl derivative which showed no NH- or OH-absorption in the IR region. The compound was purified on silica gel to yield the desacetyl derivative $C_{25}H_{31}N_5O_4$(**4**) through the loss of both acetyl groups. In the ^{1}H NMR spectrum the protons at C-13 and C-15 appeared as singlets at δ4.59 and δ6.07, respectively. The singlet at δ5.45 was assigned to the proton at C-8.

Dihydro-oxaline was stable towards treatment with OsO_4 in aqueous acetic acid or hydrogen peroxide in aqueous alkali. Oxidation of dihydro-oxaline (**2a**) with aqueous $KMnO_4$ in pyridine gave two products in very low yield. The one compound, $C_{20}H_{23}N_3O_5$(**5a**), was formed by the oxidative cleavage of the 12,15-double bond. Its ^{1}H NMR spectrum showed an ABCD pattern assigned to the four aromatic protons, the methoxy groups at δ3.62 and δ3.72 and the presence of the reduced isoprene side-chain. The 8-H appeared at δ5.39. The IR spectrum showed absorption at 3395(N–H) 1800, 1765 and 1705 cm^{-1}. The strong absorption at 1800 and 1765 cm^{-1} was assigned to the CO absorption of ring 4 in **5a**. The other compound, $C_{16}H_{21}N_3O_2$(**6**) was formed from the oxidation of ring 3. Its ^{1}H NMR spectrum supported the presence of the part structure as shown. However, the low yield of this compound precluded a complete structural assignment. The oxidation of N-methyldihydro-oxaline with aqueous $KMnO_4$ under similar conditions gave compound **5b** as the only isolable product. Its IR spectrum was virtually identical to that of **5a** except for the lack of NH absorption and in its ^{1}H NMR spectrum the N-methyl group was evident by absorption at δ2.60.

Oxaline contains several unique structural features. To our knowledge oxaline is the first known fungal metabolite containing the N-methoxylindoline moiety. A few plant products, e.g. neoglucobrassicin,[7] 1,5-dimethoxy-3-(dimethylaminomethylindole)[8] and lespedamin[9] contain the N-methoxylindole grouping. A further uncommon feature is the location of the isoprene unit (linked in the reverse fashion) to C-3 of tryptophan; C-2 is the common location as in the brevianamides,[5] austamides[4] and echinulins.[10] It is furthermore of importance that C-2 carries three nitrogen functionalities.

Biogenetically the basic skeleton of oxaline is clearly derived from a dioxopiperazine formed from tryptophan and histidine. During the process the dioxopiperazine ring apparently undergoes an oxidative cleavage which can lead to the uncommon linking of the tryptophan and histidine moieties in oxaline. Since our previous com-

munication,[2] Scott *et al.*[11] reported the isolation of roquefortine, a biogenetically related metabolite.

^{13}C NMR *study*

The assigned proton-noise-decoupled (p.n.d.) natural-abundance ^{13}C NMR spectrum of oxaline (**1a**) is shown in Fig. 1. The ^{13}C data for oxaline and some of its derivatives, obtained from p.n.d. and NOE enhanced single frequency spectra, are given in Table 1.

In the assignment of the ^{13}C NMR spectrum of oxaline (**1a**) use has been made of chemical shift values of related compounds, the observed directly bonded ($^1J_{CH}$), long-range (over more than one bond) ($^{>1}J_{CH}$), carbon-13-proton coupling constants and of techniques such as off-resonance proton decoupling, selective population inversion (SPI)[12] and difference selective population inversion (DSPI).[13]

Off-resonance proton decoupling experiments determined the resonances which arose from methyl (four), methylene (one), methine (nine) and quaternary (ten) C atoms. Chemical shifts and directly bonded C–H coupling constants distinguished between the Me and OMe resonances. The signals due to the two Me carbons of the isoprene side-chain appeared at δ24.1 and δ23.7. The OMe carbon signals are at δ55.7 and δ65.2 with the latter characteristic for an N–OMe carbon.[14] The only methylene carbon (C-23) resonates at δ113.9.

By correlating the residual splittings[3] in off-resonance proton decoupled spectra with the known proton chemical shifts, the signals at δ142.8, δ109.7 and δ107.0 could be assigned to C-22, C-15 and C-8, respectively. Four methine carbon signals with chemical shifts (δ112.0–128.4) and directly bonded C–H coupling constants were characteristic of aromatic C atoms. The resonances at δ124.7 and δ112.0 were similarly correlated[3] with the two "doublets" (δ_H7.60 and δ_H6.92, respectively) and the resonances at δ128.4 and δ123.3 with the two "triplets" (δ_H7.09 and δ_H7.23, respectively) in the aromatic proton spectrum. These resonances have been assigned to C-4, C-5, C-6 and C-7 (Table 1) by using the reported ^{13}C chemical shifts of related compounds.[15] This information facilitated the unambiguous assignment of the aromatic proton resonances (see before).

The assignment of the remaining methine carbon signals at δ136.6 ($^1J_{CH}$ = 208 Hz) to C-18 and δ133.8 ($^1J_{CH}$ = 190 Hz) to C-20, is based on the observed C–H coupling constants. In imidazole[16] the directly bonded C–H coupling constants ($CDCl_3$ solution) for the corresponding methine carbon atoms are 205.1 Hz and 188.6 Hz, respectively. Similar values are observed in substituted purines.[17]

Two of the aliphatic quaternary C atoms have been assigned from chemical shift considerations. The peak at δ52.6 has been attributed to C-3, this C atom is next to a double bond and a phenyl group while C-21 (only adjacent to a double bond) resonates at δ42.6. To assign the low-field quaternary carbons in oxaline long-range C–H coupling constants were used. The single frequency NOE enhanced ^{13}C spectrum of this region is depicted in Fig. 2(a). The two resonances at lowest field, viz δ166.1 and δ157.3 can be attributed to the amide carbonyl C atoms (C-10 and C-13). Most of these quaternary carbons appeared as doublets except for the resonance at δ126.0 (singlet) and the resonances at δ146.6 and δ146.5 (triplets).

In the assignment of the eight low-field resonances SPI[12] and DSPI[13] have been used. The results are shown in Fig. 2. In aromatic systems $^3J_{CH}$ is normally in the order of 8 Hz while $^2J_{CH}$ is small.[18] By the selective inversion of a C-4 proton transition, the spectrum shown in Fig. 2(d) is obtained in the difference mode (DSPI). This affected the signals attributed to C-6 and the outer legs of the triplet at δ146.6, therefore, assigning it to C-7a. Similarly the

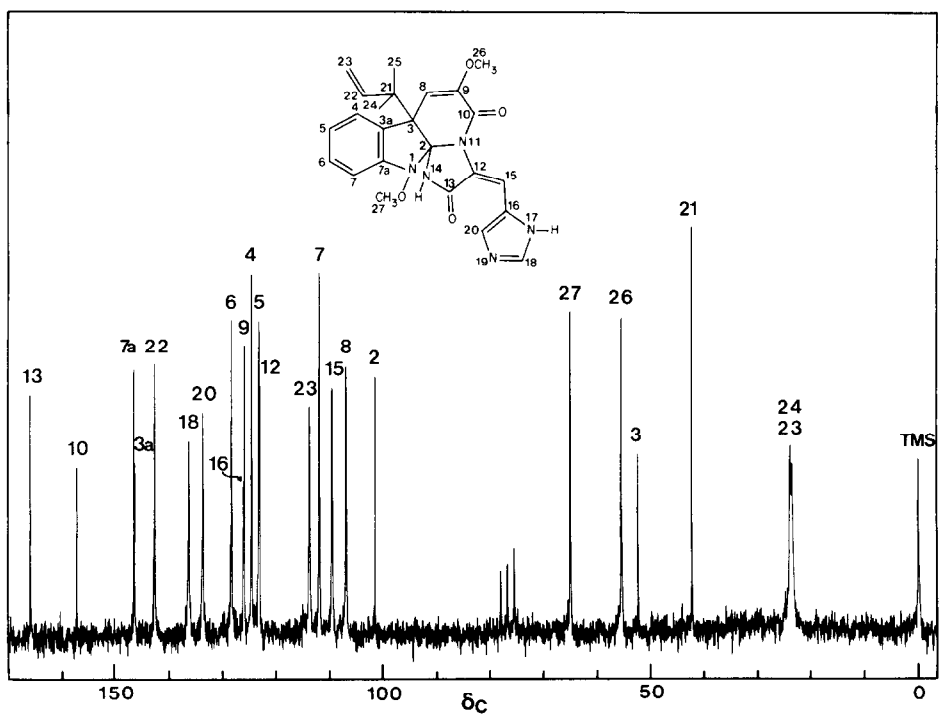

Fig. 1. The natural-abundance p.n.d. 25.2 MHz ^{13}C NMR spectrum of oxaline (**1a**). Spectral width = 5 KHz; pulse delay: 0.4 sec; 90° pulse; transients 3 K.

Table 1. ^{13}C-Chemical shifts, directly bounded ($^1J_{CH}$) and long range ($^{>1}J_{CH}$) carbon-13-proton coupling constants (J in Hz) of oxaline (**1a**) and its derivatives

Carbon	Oxaline (1a)			N(19)-Acetyloxaline (1c)			N(14)-Methyl oxaline (1b)	Dihydro-N(19)-acetyl oxaline (2d)	Hexahydro-N,N'-diacetyl oxaline (3)
	δ (ppm)*	$^1J_{CH}$	$^{>1}J_{CH}$	δ (ppm)*	$^1J_{CH}$	$^{>1}J_{CH}$	δ (ppm)*	δ (ppm)*	δ (ppm)*
2	101.6 Sd	-	6	100.9 Sd	-	6	104.1	101.5	100.8
3	52.6 Sm	-		52.3 Sm	-		53.6	53.9	53.8
3a	146.5 St	-	4	146.3 St	-	4	146.0	146.6	146.0
4	124.7 Dd	160	7	124.6 Dd	160	7	123.6	125.2	124.9
5	123.3 Dd	162	7	123.3 Dd	162	7	123.1	123.4	123.4
6	128.4 Dd	161	7	128.3 Dd	161	7	128.7	128.5	128.4
7	112.0 Dd	165	8	112.0 Dd	165	7	110.1	112.2	112.0
7a	146.6 St	-	8	146.4 St	-	8	147.1	146.8	146.3
8	107.0 D	164	-	107.1 D	164	-	107.9	107.8	108.4
9	126.0 S	-	-	125.9 Sm	-		125.2	123.4†	125.9†
10	157.3 Sd	-	8	157.1 Sd	-	9	157.7	157.7	157.5
12	123.1 Sd	-	7	126.4 Sd	-	7	122.5	126.6†	127.3†
13	166.1 Sd	-	10	163.8 Sd	-	10	165.3	164.8	164.3
15	109.7 D	161	-	114.3 D	162	-	109.2	114.9	119.6
16	126.2 Sd	-	13	137.3 Sddd	-	4;8;12	**	137.6	50.7
18	136.4 Dd	208	12	135.2 Dd	215	6	136.4	135.5	60.5
20	133.8 Ddd	190	3;9	119.6 Dd	202	8	134.5	120.0	51.2
21	42.5 Sm	-		42.3 Sm	-		42.0	40.4	40.4
22	142.8 Dm	149		142.6 Dd	149		142.6	~ 29	30.0
23	113.9 DDd	153;159	3	114.1 Tm	156		113.6	8.2	8.2
24	24.1 Qm	125		24.0 Qm	125		24.8	~ 22	21.9
25	23.7 Qm	125		23.8 Qm	125		23.1	~ 22	21.8
26	55.7 Q	145	-	55.7 Q	145	-	55.7	55.6	55.6
27	65.2 Q	144	-	65.0 Q	144	-	65.1	65.2	65.1
C=O				166.4 Sq	-	7		167.7	168.8
									168.2
CH_3				22.9 Q	130	-		22.9	22.2
									21.5
N-CH_3							32.3		

*Chemical shifts relative to internal Me_4Si. Capital letters refer to the pattern resulting from directly bonded protons and small letters to long range C–H coupling. S = singlet, D or d = doublet, T or t = triplet, Q or q = quartet and m = multiplet.

**Not observed.

†May be interchanged.

resonance at δ146.5 has been assigned to C-3a by the selective inversion of a C-7 proton transition. On the assumption that three bond C–H coupling constants are generally larger than two bond couplings the following assignments have been made: The inversion of the high-field transition of the C-8 proton affected the resonances at δ157.3 and δ101.6 (Fig. 2(b)) thereby assigning the former to C-10 and the latter to C-2. The other amide carbonyl atom resonance (δ166.1) must, therefore, be attributed to C-13. The remaining quaternary carbons (C-9, C-12 and C-16) appeared as a doublet ($^{>1}J = 7$ Hz) at δ 123.1, a doublet ($^{>1}J = 13$ Hz) at δ 126.2 and a singlet at δ126.0. In imidazole[16] $^2J_{C4H5} = {}^2J_{C5H4} =$ 13.4 Hz; these values correspond to the coupling observed for the peaks at δ126.2; this evidenced for the assignment of this resonance to C-16. The inversion of the low-field transition of the C-15 proton affected the resonances at δ166.1 (C-13) and δ123.1 (Fig. 2(c)). The long-range carbon-13-proton coupling observed in latter resonance has, because of its magnitude been attributed to a two bond coupling between C-12 and 15-H, assigning the resonance to C-12. The only remaining singlet (δ126.0) is assigned to C-9.

Further support for the above assignment is obtained from the observed chemical shifts of the derivatives of oxaline as given in Table 1. In N(14)-methyloxaline (**1d**) a pronounced shift is observed for C-2 only. The chemical shifts of C-15 and C-16 in N(19)-acetyloxaline (**1c**) differ markedly from those in oxaline and distinguish clearly between C-8 and C-15 and between C-12 and C-16.

The additivity of substituent effects[19] are frequently assumed in the assignment of ^{13}C resonances in p.n.d. spectra. Oxaline provides a good example to test the applicability of these additivity rules to aryl shielding in a complex molecule. The experimental chemical shift values for the aromatic carbon atoms in oxaline are compared in Table 2 to the corresponding calculated values[20] and to some observed results for related compounds.

The values calculated from the shift increments for monosubstituted benzenes[20] (Table 2, columns 2 and 3)

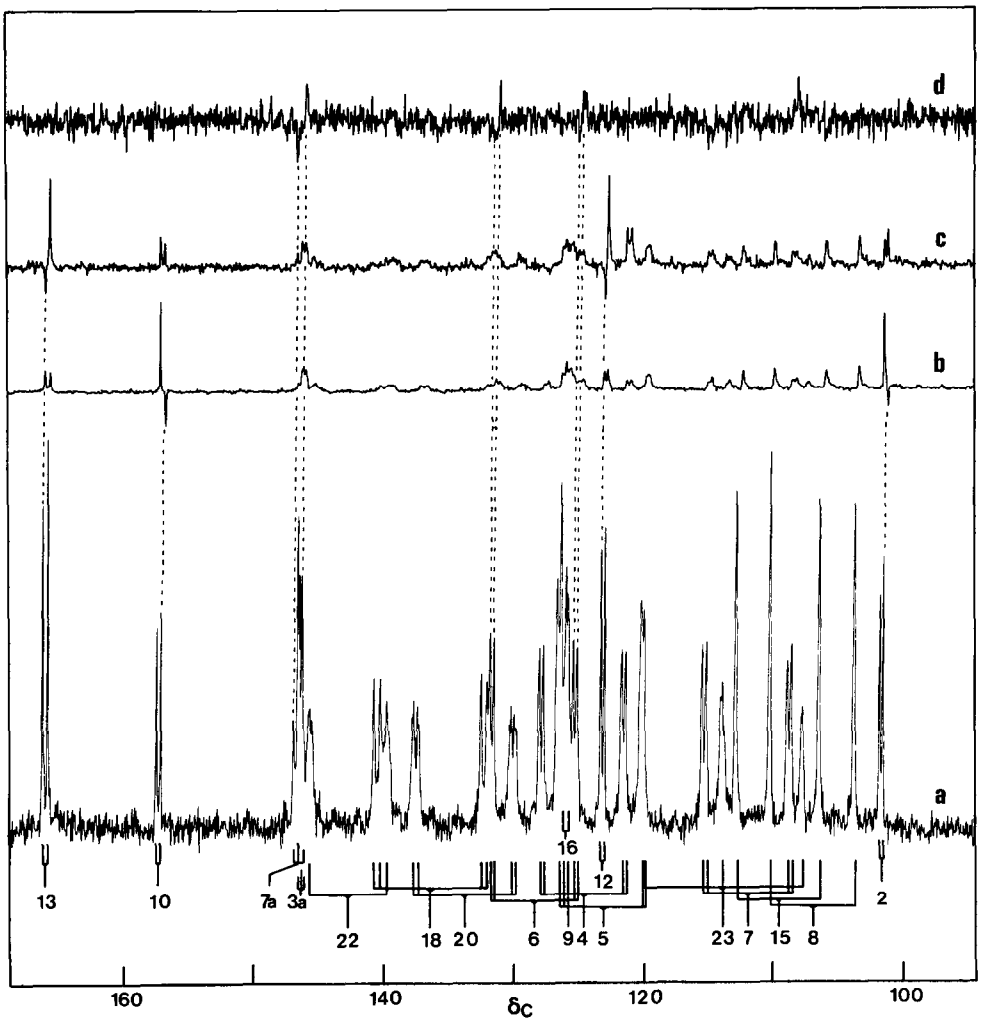

Fig. 2. (a) Part of the natural-abundance coupled NOE 25.2 MHz ^{13}C spectrum of oxaline (**1a**). Total spectral width: 4 KHz; 90° pulse; transients: 15.9 K; decoupler on time: 2 sec. (b) Result of an SPI experiment. High-field transition of C-8 proton selectively inverted. π-pulse: 0.08 sec. (c) Result of an SPI experiment. Low-field transition of C-15 proton selectively inverted. π-pulse: 0.08 sec. (d) Result of a DSPI experiment. High-field transition of C-4 proton selectively inverted. π-pulse: 0.16 sec.

Table 2. The observed and calculated ^{13}C chemical shifts of the aromatic carbons of oxaline (**1a**) and some related compounds

Carbon	1 Oxaline	2[a] CH_3 NH_2	3[a] $C(CH_3)_3$ $N(CH_3)_2$	4[b] o-CH_3 $N(CH_3)_2$	5[c] o-$C(CH_3)_3$ $N(CH_3)_2$	6[b] Vindolinine	7[b] Oxindole alkaloids
3a	146.5	125.4	134.9	132.3	145.1	139.8	134.1-134.4
4	124.7	130.4	125.9	132.0	128.0	123.6	122.1-123.0; 125.1-125.2
5	123.3	119.0	116.3	124.0	123.6	121.0	122.1-122.6
6	128.4	126.7	126.2	127.1	127.1	127.2	127.4-128.0
7	112.0	116.1	112.4	119.8	119.4	112.0	109.6-109.8
7a	146.6	148.3	147.5	153.9	149.9	149.4	140.7-141.7

[a]Values calculated from the shift increments of the two monosubstituted benzenes indicated.
[b]Observed values: column 4, Ref. 21; column 6, Ref. 22; column 7, Ref. 15.
[c]The difference of the shift increments for a *t*-butyl and methyl group added to the values of column 4.

differ markedly from certain of the observed aromatic carbon chemical shifts of oxaline. Although column 3 produces the correct sequence these values cannot be used for assignment purposes. This agrees with the finding that deviations from additivity are common in *ortho*-substituted systems, especially in the case of substituents containing unshared electrons or double bonds.[19,21] To take the *ortho*-effect into account the determined chemical shifts of N,N'-dimethyl-*o*-toluidine[21] (Table 2 column 4) have been used as a basis. The addition of the difference between the shift increments of a *t*-butyl and a methyl group to these values (Table 2 column 5) resulted in a better agreement between observed and calculated shifts (average deviation 2.8 ppm). According to these values the assignment of C-4 and C-6 should have been interchanged in oxaline. It is obvious from the calculated chemical shifts in Table 2 that extreme care should be taken when additivity relationships are used for the assignment of closely spaced ^{13}C resonances. The use of chemical shifts of related compounds[15,22] is a more reliable approach. The values for the different proton bearing carbon atoms of the model compounds (Table 2, columns 6 and 7) and of oxaline are all within a narrow range.

N-Acetylation of the imidazole moiety in oxaline affects the chemical shifts and one-bonded C–H couplings in that part of the molecule considerably. Chemical shifts may be influenced by remote substituents as well as by conformational changes while directly bonded couplings are dominated by the Fermi contact term which reflects the electronic structure of the bond. Both one bond C–H couplings in the imidazole ring change considerably [Δ^1J(C-18) = −7 Hz, Δ^1J(C-20) = −12 Hz], suggesting that N-acetylation has occurred in the 19-position. Changes of comparable magnitude in the ^1J-values (−13 Hz and −12 Hz, respectively) have been observed for imidazole and histidine[23] in going from the neutral to the cationic form. This effect has been attributed to changes in the electronic structure of the imidazole ring following protonation. A similar explanation cannot be forwarded for the acetylated species and the similarity in the change of coupling constants may be incidental. The phenomenon of the observed change in coupling constants upon N-acetylation of the imidazole moiety, probably attributable to electron withdrawal, is under investigation.

EXPERIMENTAL

UV absorption refers to MeOH and IR absorption to $CHCl_3$. UV spectra (Unicam Model S.P. 800 spectrometer) and IR spectra (Perkin–Elmer Model 237 spectrometer). Mass spectra were taken on an MS-9 double focussing mass spectrometer. ^1H NMR spectra were recorded on a Varian HA-100 spectrometer in $CDCl_3$. ^{13}C NMR spectra were recorded on a Varian XL-100-15 FT spectrometer in $CDCl_3$. TLC chromatography was carried out on Merck precoated SiO_2 plates, layer thickness 0.25 and 1.25 mm for analytical and preparative separations, respectively. Chromogenic agent for TLC plates was a soln of 1% $Ce(SO_4)_2$ in 6N H_2SO_4.

Isolation of oxaline (**1a**). *P. oxalicum* strain CSIR M-555 was grown in bulk on wet sterilized maize meal for 21 days. The dried maize meal (5 kg) was extracted with $CHCl_3$–MeOH over a period of 3 days and the solvent removed under reduced pressure to yield an insoluble fraction (105 g), representing secalonic acid D and a soluble fraction (340 g). The latter in $CHCl_3$ (4 l) was twice extracted with water (2 l). Evaporation of the $CHCl_3$ yielded 310 g of material which was partitioned between 90% MeOH and hexane (3 l each). Work-up of the MeOH fraction yielded 35 g of material which was separated by chromatography on silica gel (2 kg). Oxaline (1.5 g) was eluted with $CHCl_3$: MeOH (95 : 5, v/v).

Oxaline crystallised from nitromethane or $CHCl_3$, m.p. 220–221°. It had $[\alpha]_D^{22}$ −45° (*c*, 0.3 : MeOH); c.d. (MeOH) $\Delta\epsilon_{420}$ 0, $\Delta\epsilon_{343}$ −7.8, $\Delta\epsilon_{309}$ 0, $\Delta\epsilon_{275}$ +13.05, $\Delta\epsilon_{259}$ +11.60, $\Delta\epsilon_{247}$ +13.75, $\Delta\epsilon_{237}$ 0, $\Delta\epsilon_{223}$ −32.25 and $\Delta\epsilon_{208}$ 0; λ_{max} 228 and 347 nm (log ϵ 4.32 and 4.39, respectively); ν_{max} 3425 [N(14)-H], 3182 (N–H, imidazole), 2985, 2942, 1710, 1705, 1686 and 1640 cm^{-1}.

The high resolution mass spectrum showed *m/e* 447.1890 (M^+, $C_{24}H_{25}N_5O_4$ requires: 447.1906), 379.1276 ($C_{19}H_{17}N_5O_4$ requires: 379.1280). [Found: C, 64.25; H, 5.70; N, 15.68. $C_{24}H_{25}N_5O_4$ requires: C, 64.15; H, 5.60; N, 15.65%].

The permethylation of oxaline **1a**. Oxaline (**1a**; 20 mg) in dry DMSO (1.0 ml) was slowly added to a suspension of 50% NaH (6 mg) and DMSO (2 ml) and stirred at room temp. in an atmosphere of dry N_2 for 30 min. MeI (40 mg) was added to the mixture and stirred for a further 30 min. The mixture was poured onto ice and extracted into $CHCl_3$. The organic layer was separated by SiO_2 TLC in $CHCl_3$: MeOH : acetone 92 : 4 : 4 (v/v/v) yielding **1b** (9 mg) at R_f 0.45. The glass had ν_{max} 1720, 1690 and 1650 cm^{-1} and *m/e* 475.222 (M^+, $C_{26}H_{29}N_5O_4$ requires: 475.222).

The same compound (**1b**) can be obtained upon treatment of oxaline in $CHCl_3$ with Ag_2O/MeI.

Dihydro-oxaline (**2a**). Oxaline **1a** (400 mg) was hydrogenated in AcOH (50 ml) over PtO_2 (200 mg). After uptake of 1 mol of H_2 during 2 hr the absorption ceased. The mixture was filtered through celite and the filtrate evaporated to give **2a** (390 mg) m.p. 254–255° ($CHCl_3$-ether). It had λ_{max} 228 and 347 nm (log ϵ 4.29 and 4.37, respectively); ν_{max} 3420 3185, 1710, 1705, 1685 and 1635 cm^{-1}; *m/e* 449.205 (M^+, $C_{24}H_{27}N_5O_4$ requires: 449.206), 379.127 ($C_{19}H_{17}N_5O_4$ requires: 379.128). [Found: C, 64.39; H, 6.20; N, 15.50. $C_{24}H_{27}N_5O_4$ requires: C, 64.15; H, 6.01; N, 15.60%].

N(19)-*Acetyldihydro-oxaline* (**2d**). Dihydro-oxaline **2a** (100 mg) in pyr.: Ac_2O 1 : 1 (10 ml) was left at room temp. for 4 hr. Work-up as for **1c** gave **2d** (104 mg). *m/e* 491, (M^+, $C_{26}H_{29}N_5O_5$ requires: 491).

N(19)-*Acetyloxaline* (**1c**). Oxaline **1a** (100 mg) in pyr.: Ac_2O 1 : 1 (10 ml) was left at room temp. for 4 hr. The reagents were removed in a stream of dry N_2 and the residue partitioned between $CHCl_3$ and water to give **1c** (105 mg) as an oil. It had ν_{max} 3425 [N(14)-H], 1739 (strong), 1693 and 1639 cm^{-1}; *m/e* 489.203 (M^+, $C_{26}H_{27}N_5O_5$ requires: 489.203).

N(19)-acetyloxaline is a labile compound and can be converted into oxaline (m.p. 220°) by filtration through silica gel in $CHCl_3$: MeOH : acetone (95 : 3 : 2 v/v/v) as eluent.

Hexahydro-N,N'-*diacetyloxaline* (**3**). Oxaline **1a** (400 mg) in $(Ac)_2O$: AcOH (1 : 1) (50 ml) was shaken in an H_2 atmosphere over PtO_2 (400 mg) for 36 hr. The course of the reaction was monitored by the disappearance of the long wavelength UV absorption (347 nm). The mixture was filtered and the residue separated on preparative SiO_2 TLC in $CHCl_3$: MeOH 92 : 8 (v/v) to give **3** (250 mg), m.p. (227–228° (acetone–ether). It had λ_{max} 238 and 288 (log ϵ 4.24 and 4.00, respectively); ν_{max} 3410 [N(14)-H], 1730, 1690, 1655, 1650 and 1635 cm^{-1}; *m/e* 537 (M^+, $C_{28}H_{35}N_5O_6$ requires: 537), 467.1803 ($C_{23}H_{25}N_5O_6$ requires: 467.1805). [Found: C, 62.45: H, 6.47. $C_{28}H_{35}N_5O_6$ requires: C, 62.55: H, 6.56%].

A minor reaction product (15 mg) representing octahydro-N,N'-diacetyloxaline, $C_{28}H_{37}N_5O_6$, was isolated from the silica chromatoplates. It has *m/e* 539 (M^+, $C_{28}H_{37}N_5O_6$ requires: 539), 469.199 ($C_{23}H_{27}N_5O_6$ requires: 469.196).

Monobromodihydro-oxaline (**2b**). Dihydro-oxaline **2a** (140 mg) in THF (30 ml) was treated with pyridinium hydrobromide perbromide (230 mg) in THF (10 ml) at room temp. over a period of 30 min. The mixture was stirred for a further 2 hr and poured onto ice and extracted into $CHCl_3$. The residue was separated on preparative SiO_2 TLC in $CHCl_3$: MeOH 94 : 6 (v/v) and yielded **2b** (160 mg) as the main product, m.p. 220° (acetone). It had λ_{max} 228, 282 and 350.5 nm (log ϵ 4.36, 2.86 and 4.44, respectively); ν_{max} 3410, 3175, 1700, 1680 and 1635 cm^{-1}); *m/e* 527 and 529 (1 : 1) [M^+, $C_{24}H_{26}N_5BrO_4$ requires: 527 and 529 (1 : 1)]. [Found: C, 54.15; H, 4.95; N, 13.08; Br, 15.13. $C_{24}H_{26}N_5BrO_4$ requires: C, 54.55; H, 4.93; N, 13.25; Br, 15.13%].

N(14)-*Methyloxaline* (**1d**). Oxaline **1a** (200 mg) in $CHCl_3$ (60 ml) was treated with an excess of ethereal diazomethane for 16 hr. The reagents were removed in a stream of N_2 and the residue separated by chromatography on silica gel. Elution with $CHCl_3$: MeOH : acetone 96 : 2 : 2 (v/v/v) gave **1d** (210 mg), m.p. 214–216° ($CHCl_3$-ether). It had ν_{max} 3200 (N–H, imidazole), 1700,

1690 and 1645 cm^{-1}; *m/e* 461.205 (M^+, $C_{25}H_{27}N_5O_4$ requires: 461.205) 393.141 ($C_{20}H_{19}N_5O_4$ requires: 393.141).

N(14) - *Methyldihydro - oxaline* (**2c**). N-methyloxaline **1d** (100 mg) was hydrogenated in AcOH (30 ml) over PtO_2 (30 mg). Absorption of H_2 (1 mol) ceased after 2 hr. Standard workup gave **2c** (102 mg). It had m.p. 218–220° ($CHCl_3$–ether), λ_{max} 224 and 348 nm (log ϵ 4.21 and 4.27, respectively), ν_{max} 3200 (N–H, imidazole) 1690, 1680 and 1640 cm^{-1}, *m/e* 463 (M^+, $C_{25}H_{29}N_5O_4$ requires: 463). [Found: C, 64.48; H, 6.55; N, 14.85. $C_{15}H_{29}N_5O_4$ requires: C, 64.78; H, 6.31; N, 15.11%].

The LAH *reduction of* N(14)-*methyldihydro-oxaline* (**2c**). N-methyldihydro-oxaline **2c** (20 mg) in dry THF (4 ml) was treated at room temp. for 5 min with LAH (10 mg). The mixture was poured onto ice containing NH_4Cl and extracted into $CHCl_3$. The organic residue (19 mg) in pyr.: Ac_2O 1:1 (4 ml) was left at room temp. for 16 hr. The reagents were removed in a stream of dry N_2 and the residue partitioned between $CHCl_3$ and water to give an oil (21 mg). Its ^1H NMR and IR spectra were recorded immediately. Its IR spectrum showed no NH or OH bands and ν_{max} 1735, 1705, 1675 and 1648 cm^{-1}. It had *m/e* 549 (M^+, $C_{29}H_{35}N_5O_6$ requires: 549) and 447 [$M^+ - (2CH_2CO + H_2O)$ requires: 447].

The above compound decomposed and was separated by SiO_2 TLC in $CHCl_3$: MeOH 92:8 (v/v). The main band R_f 0.40 was eluted with MeOH to give **4** (12 mg). It had ν_{max} 3420 (br), 3195, 1682 and 1642 cm^{-1}, *m/e* 465 (M^+, $C_{25}H_{31}N_5O_4$ requires: 465) and 447 ($C_{25}H_{29}N_5O_3$ requires: 447).

$KMnO_4$ *oxidation of dihydro-oxaline* (**2a**). $KMnO_4$ (400 mg) in water (6.5 ml) was added dropwise over a period of 45 min to **2a** (170 mg) in pyr. (30 ml) at 88°. Stirring was continued for a further 5 min. The mixture was filtered and the pyr. removed from the filtrate at low temp. The residue was partitioned between $CHCl_3$ and water. The $CHCl_3$ yielded a complex mixture (77 mg) which was separated by SiO_2 TLC in $CHCl_3$: MeOH 93:7 (v/v) to give **6** R_f 0.5 (12 mg) as a glass. It had ν_{max} 3400 (broad) 1720 and 1664 cm^{-1}; *m/e* 287.1612 ($C_{16}H_{21}N_3O_2$ requires: 287.1633), 189.0906 ($C_{10}H_{11}N_3O$ requires: 189.0902), 173.0712 ($C_{10}H_9N_2O$ requires: 173.0714), 143.0582 ($C_9H_7N_2$ requires: 143.0609). The less polar compound **5a** (8 mg) R_f 0.75 also failed to crystallise. It had λ_{max} 233 and 285 nm; ν_{max} 3395, 1800(s), 1763, 1708 and 1630 cm^{-1}; *m/e* 385 (M^+, $C_{20}H_{23}N_3O_5$ requires: 385).

$KMnO_4$ *oxidation of* N-*methyldihydro-oxaline* (**2c**). N-methyldihydro-oxaline **2c** (45 mg) was similarly treated with aqueous $KMnO_4$ (80 mg). Standard work-up gave the N–Me derivative **5b** (6 mg) as an amorphous powder. It had λ_{max} 233 and 285 nm (log ϵ 4.21 and 3.79, respectively); ν_{max} 1800, 1748, 1710 and 1630 cm^{-1}; *m/e* 399.1783 (M^+, $C_{21}H_{25}N_3O_5$ requires: 399.1794).

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REFERENCES

[1]P. S. Steyn, *Tetrahedron* **26**, 51 (1970).
[2]D. W. Nagel, K. G. R. Pachler, P. S. Steyn, P. L. Wessels, G. Gafner and G. J. Kruger, *J. Chem. Soc. Chem. Comm.* 1021 (1974).
[3]K. G. R. Pachler, *J. Magn. Resonance* **7**, 442 (1972).
[4]P. S. Steyn, *Tetrahedron* **29**, 107 (1973).
[5]A. J. Birch and J. J. Wright, *Ibid.* **26**, 2329 (1970).
[6]K. Hofmann, *The Chemistry of Heterocyclic Compounds*, Part I, p. 111. Interscience, New York (1953).
[7]R. Gmelin and A. I. Virtanen, *Acta Chem. Scand.* **16**, 1378 (1962).
[8]S. R. Johns, J. A. Lamberton and J. L. Occolowitz, *Aust. J. Chem.* **20**, 1737 (1967).
[9]H. Morimoto and H. Oshio, *Liebigs Ann. Chem.* 681, 212 (1965).
[10]M. Barbetta, G. Casnati, A. Pochini and A. Selva, *Tetrahedron Letters* 4457 (1969).
[11]P. M. Scott, M. A. Merrien and J. Polonsky, *Experientia* **32**, 140 (1976).
[12]K. G. R. Pachler and P. L. Wessels, *J. Magn. Resonance* **12**, 337 (1973).
[13]A. A. Chalmers, K. G. R. Pachler and P. L. Wessels, *Ibid.* **15**, 415 (1974).
[14]M. Acheson, Personal communication.
[15]E. Wenkert, J. S. Bindra, C. Chang, D. W. Cochran and F. M. Schnell, *Acc. Chem. Res.* **7**, 46 (1974).
[16]M. C. Thorpe and W. C. Coburn, *J. Magn. Resonance* **12**, 225 (1973).
[17]M. C. Thorpe, W. C. Coburn and J. A. Montgomery, *Ibid.* **15**, 98 (1974).
[18]G. C. Levy and G. L. Nelson, *Carbon-13 Nuclear Magnetic Resonance for Organic Chemists*, p. 100. Wiley-Interscience, New York (1972).
[19]J. B. Stothers, *Carbon-13 NMR Spectroscopy*. Academic Press, New York (1972).
[20]J. T. Clerc, E. Pretsch and S. Sternhell, *^{13}C-Kernresonanzspektroskopie*, Akademische Verlagsgesellschaft, Frankfurt am Main (1973).
[21]P. C. Lauterbur, *J. Chem. Phys.* **38**, 1415 (1963).
[22]A. Ahond, M.-M. Janot, N. Langlois, G. Lukacs, P. Potier, P. Rasoanaivo, M. Sangare, N. Neuss, M. Plat, J. Le Men, E. W. Hagaman and E. Wenkert, *J. Am. Chem. Soc.* **96**, 633 (1974).
[23]M. W. Hunkapiller, S. H. Smallcombe, D. R. Whitaker and J. H. Richards, *Biochem.* **12**, 4732 (1973). R. E. Wasylishen and G. Tomlinson, *Biochem. J.* **147**, 605 (1975).