601. *The Chemistry of Fungi. Part XXXVI.* A Revised Structure for Sclerotiorin.*

By F. M. Dean, J. Staunton, and W. B. Whalley.

Unequivocal evidence has been obtained that sclerotiorin contains an acetoxyl group. This fact, together with more detailed studies of the properties of *N*-methylsclerotioramine and of certain spectroscopic data, leads to structure (IIa) for sclerotiorin. The new structure is in complete accord with evidence previously interpreted in terms of structure (Ia).

It has been demonstrated previously that sclerotiorin and its derivatives can be aromatised by reductive extrusion of a $C_2H_2O_2$ residue, as in the conversion [1] of sclerotioramine, $C_{21}H_{24}O_4NCl$, into aposclerotioramine $C_{19}H_{22}O_2NCl$ (XIIa). At that time it appeared that sclerotiorin had lactonic properties but did not contain an acetoxyl group, so the tentative structure (Ia) was advanced,[2] the aromatisation being assumed to involve a breakage of a carbon–carbon bond for which there are numerous precedents. It has now been proved that sclerotiorin does contain an acetoxyl group and that this pigment is correctly represented by structure (IIa).

The orientation and natures of the side-chains R in sclerotiorin (Ia or IIa), tetrahydrosclerotiorin (Ib or IIb), and dihydrosclerotiorin (Ic or IIc) have already been fully defined,[1,3,4] and it has been proved [1,2] that these side-chains are unaffected in the reactions with ammonia (or primary amines) which give the related sclerotioramines by replacement of an ether-oxygen atom. Although this type of replacement is characteristic of pyrones, the oxidation [2] of sclerotioramine to berberonic acid (IV) excludes the presence of a simple

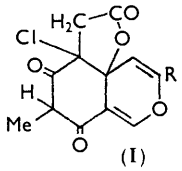
* Part XXXV, *J.*, 1958, 1833.

[1] Fielding, Graham, Robertson, Travers, and Whalley, *J.*, 1957, 4931.
[2] Fielding, Robertson, Travers, and Whalley, *J.*, 1958, 1814.
[3] Eade, Page, Robertson, Turner, and Whalley, *J.*, 1957, 4913.
[4] Graham, Page, Robertson, Travers, Turner, and Whalley, *J.*, 1957, 4924.

pyrone ring and points to the presence of a vinylogous system (extended pyrone). Moreover, these replacements occur with an ease characteristic of pyrones with acyl rather than alkyl or other substituents,[5] so that partial structures (V) and (VI) are indicated. Either of these would account for the formation of berberonic acid, the tautomeric properties of sclerotioramine [which is amphoteric and gives an *N*-methyl derivative but an *O*-acetate ($\nu_{max.}$ 1770 cm.$^{-1}$)], and the bathochromic shifts in *two* carbonyl frequencies when, for

(a) R = ·CH:CH·CMe:CH·CHMeEt (b) R = ·CH_2·CH_2·CHMe·CH_2·CHMeEt

(c) R = ·CH_2·CH_2·CMe:CH·CHMeEt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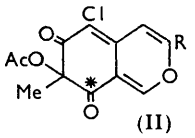
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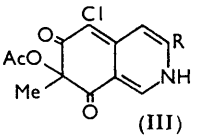
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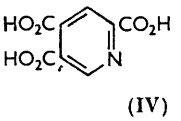
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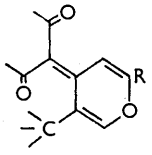
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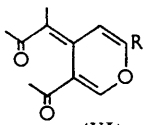
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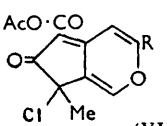
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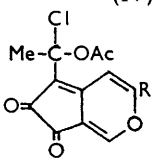
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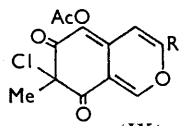
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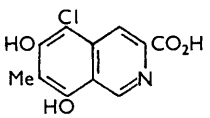
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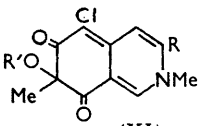
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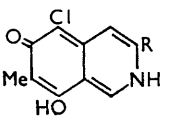

(XII)

example, tetrahydrosclerotiorin [1718 and 1640 cm.$^{-1}$ (in chloroform)] is converted into tetrahydro-*N*-methylsclerotioramine [1688 and 1600 cm.$^{-1}$ (in chloroform)].

Arrangements must now be made to accommodate in partial structures (V) and (VI) an acetoxyl group, a chlorine atom, and a methyl group which appears as acetic acid in Kuhn–Roth estimations [6] and is attached to carbon in a variety of degradation products, *e.g.*, aposclerotioraminic acid (X). Though there are many possibilities, some, *e.g.*, (VII), can be dismissed at once because sclerotiorin is obviously neither an acid chloride nor an acid anhydride, and others, *e.g.*, (VIII), can be eliminated because it is shown in the sequel that *N*-methylsclerotioramine on hydrolytic deacetylation gives an alcohol which does not easily lose hydrogen chloride, thus proving that the chlorine atom and the acetoxyl group are not attached to the same carbon atom. Of the two surviving structures, (II) and (IX), the latter can be eliminated on the grounds that the chlorine atom appears to be vinylic since it is unreactive except in conditions leading to deep-seated changes, and that the deacetylation referred to above gives an alcohol and not an enol. The conclusion that sclerotiorin has structure (IIa) is very strongly supported by the degradation [2] of sclerotioramine to aposclerotaminic acid (X), the structure of which has recently been rigidly established: [7] the opinion [2] expressed earlier, that the aromatisations do not involve skeletal rearrangements, is also justified.

It is now necessary to show that structure (IIa) accounts satisfactorily for the chief chemical properties of sclerotiorin.

[5] Ost and Reibstein, *J. prakt. Chem.*, 1881, **24**, 284; Wiley and Slaymaker, *J. Amer. Chem. Soc.*, 1956, **78**, 2393.

[6] Birch, Fitton, Pride, Ryan, Smith, and Whalley, *J.*, 1958, 4576.

[7] Staunton and Whalley, unpublished work.

That reductive aromatisation of sclerotiorin and its derivatives is necessarily effected by expulsion of a $C_2H_2O_2$ residue has always been recognised,[2,8] but the identity of this residue has been obscured by the difficulty that, on the one hand, acetic acid might be produced by retro-aldol condensations and, that on the other, formic acid has frequently been the only simple acid detectable in complex hydrolysates. Conditions are now described in which acetic acid can invariably be detected after alkaline hydrolysis of sclerotiorin. It is fortunate that, while sclerotiorin is rapidly destroyed by alkaline hydrolysis, *N*-methylsclerotioramine (IIIa; NMe for NH) is stable enough to permit selective deacetylation, giving an alcohol (XIa; R′ = H) having $\nu_{max.}$ ~3300 cm.$^{-1}$ but bereft of the ester band near 1735 cm.$^{-1}$ which characterises sclerotiorin, *N*-methylsclerotioramine, and their tetrahydro-derivatives. On acetylation, the alcohol regenerates *N*-methylsclerotioramine, so that this compound (and therefore sclerotiorin) must contain an acetoxyl group, and the name *N*-methylisosclerotioramine which was originally applied to the alcohol is now supplanted by deacetyl-*N*-methylsclerotioramine. The formation of aposclerotioramine (XIIa) when sclerotioramine is treated with zinc and alkali and the formation of the resorcinol derivative (XIII) when sclerotiorin is reduced catalytically can of course be regarded as examples of the well-known deacetoxylation of α-acetoxyketones, but a comparison with the behaviour of usnonic acid is much more impressive. Gentle oxidation of usnic acid (XIV) gives usnonic acid (XV) in which the ketol system is sufficiently stable to survive alkaline hydrolysis to the usnetic acid derivative (XVI) but which readily reverts to usnic acid when reduced by zinc.[9]

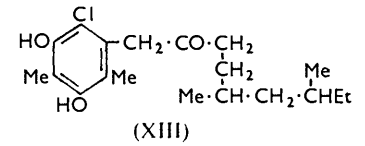
The formation of an *NO*-dimethyl derivative when sclerotioramine (IIIa) is treated with methyl sulphate and alkali [1] was formerly construed as evidence for a forced enolisation of a β-diketonic system as in (Ia) followed by alkylation. It has now been found that, short of general decomposition, *N*-methylsclerotioramine is not soluble in dilute alkali and cannot be regarded as having either an enolisable system or a lactone ring. As this alkylation cannot be effected in non-hydroxylic solvents and is accompanied by loss of absorption near 1740 cm.$^{-1}$ it must involve hydrolysis of the acetate group followed by methylation of a hydroxyl group activated by two flanking carbonyl groups. This ether is therefore not *NO*-dimethylsclerotioramine but deacetyl-*NO*-dimethylsclerotioramine (XIa; R′ = Me), its stability to dilute acids confirming the view that the hydroxyl group concerned is not enolic. The new structure also explains the production of *N*-methylaposclerotioramine (XIIa; NMe for NH) with zinc and alkali: [1] an enolic ether derived from (Ia) should have yielded an ether of *N*-methylaposclerotioramine. Similarly, tetrahydrosclerotioramine gives deacetyltetrahydro-*NO*-dimethylsclerotioramine (XIb; R′ = Me) and not tetrahydro-*NO*-dimethylsclerotioramine.

The genesis of the naphthaquinone (XXI) when tetrahydrosclerotiorin (IIb) is attacked by alkali can also be simply explained. That sclerotiorin itself dissolves in dilute sodium hydroxide and is regenerated in part by immediate acidification must now be attributed, not to the presence of a lactone ring, but to fission of the heterocyclic ring giving the enolic system (XVIIa), which eventually suffers further degradation. This interpretation is supported by the ease with which sclerotiorin forms an adduct with aniline,[3] a reaction typical of pyrones: the adduct can be represented by structure (XVIIa; NHPh for OH) in conformity with the spectroscopic properties and with the ready conversion into *N*-phenylsclerotioramine (IIIa; NPh for NH). As explained previously,[4] a similar alkaline fission of tetrahydrosclerotiorin prepares for an aldol condensation resulting in a new carbocyclic ring as in (XVIII). Aromatisation of this by a prototropic shift would give a benzyl chloride, immediate hydrolysis of which would afford a phenolic alcohol (XIX). This would enolise and suffer dehydration to the naphthaquinone (XXI) as indicated in (XX). Dihydrosclerotiorin (IIc) gives a similar naphthaquinone.

The spectroscopic properties of sclerotiorin and its derivatives can also be rationalised

[8] Birkinshaw, *Giorn. Microbiol.*, 1956, **2**, 116.
[9] Asahina and Yanagita, *Ber.*, 1938, **71**, 2260.

in terms of structure (IIa). The strong absorption at about 1730—1740 cm.$^{-1}$ of sclerotiorin, tetrahydrosclerotiorin, sclerotioramine, and related compounds is more in accord with the acetate structure (IIa) than with the strained γ-lactonic structure (Ia). Sclerotiorin and its hydro-derivatives (IIb and IIc) also absorb near 1715 and 1640 cm.$^{-1}$: the latter band is very intense and very complex so that it can be attributed to an extensively conjugated carbonyl group, but the former band seems to support a cyclopentenone grouping as in (VII) or (VIII). However, models make it clear that the strain imposed by the one tetrahedral carbon atom in the otherwise planar cyclic system of (IIa) can be relieved by twisting one carbonyl group out of the plane and therefore (to some extent) out of conjugation: the affected group might then absorb near 1715 cm.$^{-1}$. This group must be that starred in (IIa), otherwise it would be difficult to account for conjugated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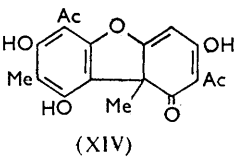
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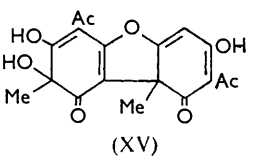
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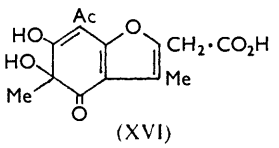
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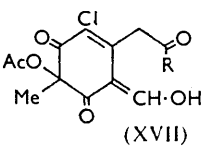
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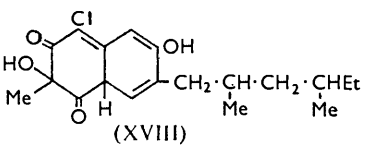
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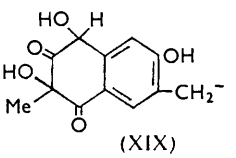
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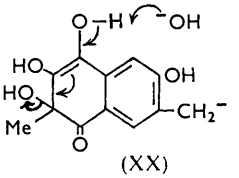
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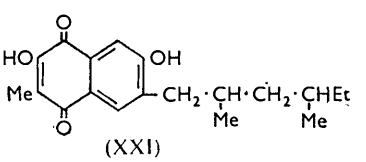
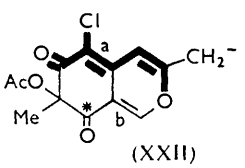

(XXI)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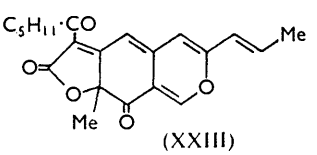
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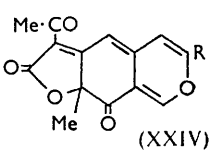
(XXIV)

carbonyl absorption as low as 1640 cm.$^{-1}$, and it is in any case improbable that the longer conjugated system would be disrupted in preference to the shorter. It must be noted that reduced conjugation in the starred carbonyl group does not vitiate the argument advanced at the beginning for the presence of an acylpyrone system, because the inductive effect alone would still constitute effective activation: in contrast, the argument would have collapsed had the other carbonyl group been displaced.

It is difficult to interpret the ultraviolet spectra of cross-conjugated systems as complex as that in sclerotiorin (IIa), but the spectrum of tetrahydrosclerotiorin (IIb) can be dealt with approximately. Again it seems that the carbonyl group starred in (II) is not effectively conjugated. Selective absorption at the longest wavelength should be defined by the system heavily marked in (XXII): the alternative system constructed with the ethylenic bond (b) would absorb at shorter wavelengths because the olefinic link (a) is now *cis*-orientated and because even a non-conjugated carbonyl group attached to (b) would reduce the π-electron mobility still further, so this system can be ignored. The parent dienone would have [10] $\lambda_{max.}$ 260 mμ, which, with increments of +20 for the two substituents

[10] Data from Braude and Nachod, " Determination of Organic Structures by Physical Methods," Academic Press, New York, 1955.

(Cl and R), +5 (exocyclic double bond), and +50 (auxochromic oxygen atom), suggests that tetrahydrosclerotiorin should have $\lambda_{max.}$ 335 mμ. This is reassuringly close to the observed value of 343 mμ, and, since the oxygen atom exerts its full auxochromic effect, it confirms the view that the starred carbonyl group is not fully conjugated. Had the other carbonyl group been displaced instead, it would have been very difficult indeed to account for intense absorption beyond about 270 mμ. The auxochromic effect of dialkylamino being ~95 mμ, a similar calculation * gives $\lambda_{max.}$ 380 mμ for tetrahydro-*N*-methylsclerotioramine (IIIb), the observed value being 381 mμ.

The lead tetra-acetate oxidation of phenols, extensively studied by Wessely and his collaborators,[11] gives acetoxycyclohexenones similar to sclerotiorin (IIa). When tetrahydro-*N*-methylaposclerotioramine (XIIb; NMe for NH) was oxidised in this way, a fair yield was obtained of a substance having the general properties of tetrahydro-*N*-methylsclerotioramine (IIIb; NMe for NH): indeed, the two materials were spectroscopically indistinguishable. Although the stereochemical complexity of the side-chain [3] and the lack of stereospecificity at the point of oxidation prevented complete identification, the nature of the reaction clearly supports structure (IIa) for sclerotiorin most strongly.

On the basis of structure (IIa), sclerotiorin is formed from a linear sequence of eight acetate units with the incorporation of three other carbon atoms, an acetoxyl group, and a chlorine atom. This accords most satisfactorily with the biogenetical evidence.[6] Finally, rubropunctatin (an extended pyrone closely similar to sclerotiorin and obtained from *Monascus rubropunctatus* Sâto) is regarded by Haws, Holker, and Kelly [12] as having structure (XXIII) and they have pointed out to us that, in the light of the revised structure for sclerotiorin, rubropunctatin can be envisaged as being biogenetically derived from a precursor similar to deacetylsclerotiorin by esterification of the tertiary hydroxyl group with β-oxo-octanoic acid followed by aldol condensation and dehydration: since rotiorin [13] is a co-pigment of sclerotiorin in *Penicillium sclerotiorum* van Beyma, similar arguments are applicable and lead to structures of type (XXIVa) for this compound.

Experimental

Alkaline Degradation of Sclerotiorin.—Sclerotiorin (2 g.) slowly dissolved when warmed on the steam-bath during 1½ hr. with 2N-sodium hydroxide solution (50 ml.). The cooled hydrolysate was acidified with excess of 2N-sulphuric acid, filtered, diluted with distilled water, and steam-distilled until the condensate was neutral. Titration with 0·1N-sodium hydroxide indicated that the total water-soluble, steam-volatile acid was equivalent to 1·59 mol.

The neutralised solution was concentrated to approx. 100 ml. and boiled for 1 hr. with an excess of 0·1N-aqueous potassium permanganate containing an excess of sodium hydrogen carbonate; then the cooled reaction mixture was acidified with 2N-sulphuric acid, filtered to remove manganese dioxide, and again steam-distilled. Titration of the distillate as above indicated the presence of 1·01 mol. of a steam-volatile acid (presumably acetic acid). Evaporation of the neutralised distillate to dryness, followed by conversion into the benzimidazole in the usual manner, gave 2-methylbenzimidazole (0·2 g.), m. p. and mixed m. p. 177° (mixed m. p. with benzimidazole *ca.* 160°). No other product could be detected.

*Deacetyl-*N*-methylsclerotioramine.*—The following improved method was used for the preparation of this compound. A solution of 2N-sodium hydroxide (5 ml.) was added to *N*-methylsclerotioramine (1 g.) in alcohol (20 ml.), and 90 seconds later the mixture was poured into water (200 ml.). The resulting precipitate was extracted with chloroform (4 × 20 ml.). The washed and dried extract was purified by chromatography on neutralised aluminium oxide and the eluate evaporated *in vacuo*, to furnish *deacetyl-*N*-methylsclerotioramine* which separated

* It will be noted that the double bond (b) is not regarded as a substituent. Justification for this rests partly on general grounds but mainly on the fact that with citrinin, which contains a similar grouping, calculations are successful only if an equivalent assumption is made.

[11] Wessely and Sinwel, *Monatsh.*, 1950, **81**, 1055, and subsequent papers.
[12] Haws, Holker, and Kelly, personal communication.
[13] Jackman, Robertson, Travers, and Whalley, *J.*, 1958, 1825.

from aqueous methanol in violet needles (0·35 g.), m. p. 160—165° (decomp.) (Found: C, 65·9; H, 6·7; N, 3·5. $C_{20}H_{24}O_3NCl$ requires C, 66·6; H, 6·7; N, 3·9%).

Reacetylation of this compound (0·35 g.) in acetic anhydride (2 ml.) containing toluene-*p*-sulphonic acid (0·3 g.) during 18 hr. at room temperature gave *N*-methylsclerotioramine (0·21 g.) in red needles (from methanol), m. p. and mixed m. p. 230—231° (decomp.), having the requisite infrared spectrum (Found: C, 65·2; H, 6·5; N, 3·4. Calc. for $C_{22}H_{26}O_4NCl$: C, 65·5; H, 6·5; N, 3·5%). This substance exhibited the characteristic dimorphism:[1] on recrystallisation from aqueous methanol the red needles were converted quantitatively into the violet modification, m. p. and mixed m. p. 230° (decomp.), having the requisite infrared spectrum; the reverse transformation was also readily effected.

The infrared spectra of these two modifications are identical when determined for chloroform solutions. But contrary to our previous report [1] the spectra are not identical when determined in Nujol. When a machine of greater resolving power than that previously available to us was used, slight differences in the 10—13·5 mμ region of the spectrum were observed.

C-Methyl estimations upon tetrahydro- and deacetyltetrahydro-*N*-methylsclerotioramine were not significantly different.

Deacetyltetrahydro-NO-*dimethylsclerotioramine*.—The method previously described [1] for the preparation of this compound has been modified as follows. Dimethyl sulphate (2 ml.) was added to a vigorously agitated solution of tetrahydrosclerotioramine (1 g.) in 2N-sodium hydroxide (50 ml.), and the oily product which separated during 10 min. was isolated with chloroform (50 ml.). After dilution with light petroleum (b. p. 60—80°) (100 ml.) the extract was chromatographed on neutralised aluminium oxide when *deacetyltetrahydro*-NO-*dimethylsclerotioramine* was eluted with chloroform–light petroleum (b. p. 60—80°) (1 : 2) and separated from methanol in red plates (0·3 g.), m. p. 201° (decomp.) [the previously recorded m. p. was 188° (decomp.)] (Found: C, 66·6; H, 8·1; OMe, 7·7. $C_{20}H_{27}O_2NCl{\cdot}OMe$ requires C, 66·5; H, 7·9; OMe, 8·2%).

Acetoxylation of Tetrahydro-N-*methylaposclerotioramine*.—When lead tetra-acetate (1 g.) was added to a solution of tetrahydro-*N*-methylaposclerotioramine (1 g.) in acetic acid (20 ml.) the oxidising agent rapidly dissolved, the solution darkened, and after 15 min. crystals separated. 24 hr. later the mixture was diluted with water (100 ml.), and the red-brown precipitate extracted with chloroform. Purification of this extract by chromatography on neutralised aluminium oxide gave a product, which separated from aqueous methanol in orange plates (0·2 g.), m. p. 198° (decomp.) and mixed m. p. 201° (decomp.) (Found: C, 64·8; H, 7·3. Calc. for $C_{22}H_{30}O_4NCl$: C, 64·7; H, 7·2%). This compound is indistinguishable from tetrahydro-*N*-methylsclerotioramine by means of the crystalline form, solubility in the usual organic solvents, or infrared spectrum or ultraviolet spectrum.

Aniline Adduct of Sclerotiorin.—This compound has $\lambda_{max.}$ 228, 285, and 365 mμ (log ε 4·2, 4·3, and 4·5 respectively) and $\nu_{max.}$ 1727 (s) (acetate), 1692 (s), 1661 (s), 1637 (s), and 1621 (s) $cm.^{-1}$.

The ultraviolet absorption spectra were measured for 95% alcohol solutions with a Unicam S.P. 500 Spectrophotometer, and the infrared spectral data were obtained on a Perkin-Elmer, Model 21, Spectrophotometer by Mr. J. V. Barkley. The analyses were by Mr. C. Tomlinson and Mr. D. Newman and their associates of this Department.

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