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A NEW QUINOLONE TYPE ALKALOID FROM *HAPLOPHYLUM TUBERCULATUM* (RUTACEAE)

D. LAVIE, N. DANIELI, R. WEITMAN and E. GLOTTER

Department of Chemistry, The Weizmann Institute of Science, Rehovoth, Israel

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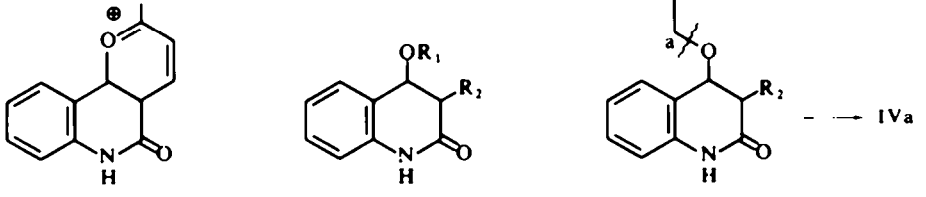
Abstract—A new alkaloid, 3-dimethylallyl-4-dimethylallyloxy-2-quinolone, has been isolated from *Haplophylum tuberculatum* (Rutaceae) and characterized by NMR and mass spectrometry as well as by conversion into dihydroflindersine. The known alkaloid flindersine has been also isolated from this plant.

AS PART of our survey of the flora of Israel, the herb *Haplophylum tuberculatum* (Rutaceae) has been more extensively investigated. The leaves and stems of the plant have been successively extracted with various solvents. Chromatography of the hexane extract yielded two crystalline nitrogen containing compounds $C_{14}H_{13}O_2N$(I) and $C_{19}H_{23}O_2N$ (II). Compound I, according to its physical constants and spectral properties, is identical with the known alkaloid flindersine[1] which is present in certain species of the Rutaceae family and its identification was accomplished by direct comparison with an authentic sample; also the dihydroderivative (III) which was obtained by catalytic hydrogenation of I was found identical with a synthetic sample of dihydroflindersine.[2]

The mass spectrum of flindersine (I; Fig. 1) is remarkably simple. Most of the total ion current is carried by two ionic species, the molecular ion *m/e* 227 (41% of the base peak) and "M-15", *m/e* 212, the base peak. The outstanding stability of the latter, formed by loss of a Me group from the molecular ion, can be understood by the formation of the fully conjugated system A:



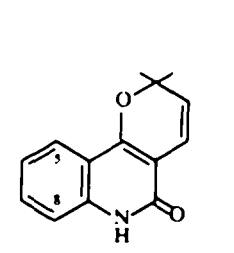
A B C

As to compound II, its UV absorption spectrum was also indicative for a 2-quinolone type chromophore,[3] λ_{max} 227, 272, 324 and 338 mμ (ε 41,300, 32,200, 8500 and 5800).

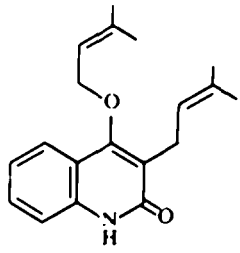
All the 23 protons of this compound could be accounted for in the NMR spectrum; the presence of four vinylic Me groups was disclosed by singlets at δ 1·70 (6 protons), 1·80 and 1·88 (3 protons each); two doublets of two protons each, centered at δ 3·50 and 4·57, accounted for the deshielded protons of two methylene groups, while

an undefined multiplet between δ 5·18–5·82 was indicative for the presence of two vinylic protons. The low field region of this spectrum could almost be superimposed on that of compounds I and III. Analogously, the complex multiplet between δ 7·03–7·55 was assigned to the aromatic 6, 7 and 8 protons, and the double doublet centered at δ 7·75 to the 5-H. This assignment is in agreement[4] with the higher deshielding of the 5-H in 4-alkoxy-2-quinolones and the isomeric 2-alkoxy-4-quinolones. Robertson[5] has shown that a distinction can be made between these two systems by using the magnitude of the relative deshielding of the 5-H: while in 4-alkoxy-2-quinolones this signal is shifted downfield from the center of the multiplet of the other three aromatic protons by less than 40 c/s, in the 2-alkoxy-4-quinolones this distance is considerably larger, about 60 to 70 c/s. The corresponding distance of 27 c/s measured in the spectrum of compound II indicates therefore a 4-alkoxy-2-quinolone type structure. Furthermore, only one signal (1-H) of an exchangeable proton appeared at extreme low field (δ 11·66) with the same chemical shift as the amidic proton of flindersine. The possibility that this signal belongs in compound II to a hydroxylic proton (at C-4) is excluded by the failure of II to yield a methyl ether with diazomethane.

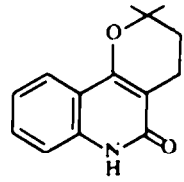
These data, in conjunction with the absorption spectrum and the IR band at 1634 cm^{-1} characteristic for an amidic CO, are suggestive for the partial structure B for compound II.



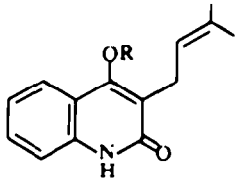
I



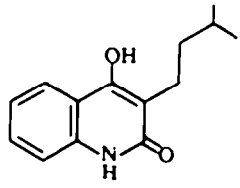
II



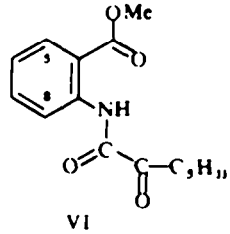
III



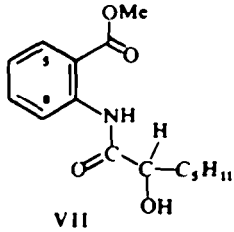
IV a R = H
b R = Me



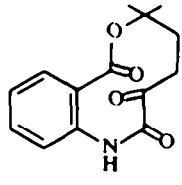
V



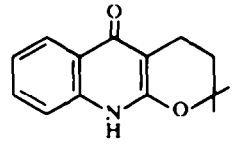
VI



VII



VIII



IX

In the mass spectrum of compound II (Fig. 2) the base peak at *m/e* 228 is formed by loss of a fragment of 69 mass units from the molecular ion (*m/e* 297) and it is accompanied by a peak at *m/e* 229 formed by loss of 68 m.u. The other two major peaks at *m/e* 174 and *m/e* 186 are also most prominent in the spectra of dihydroflindersine (III; Fig. 3) and khaplofoline[6] (IX). This may indicate that in the ions *m/e* 228 and 229 the two O atoms are retained and consequently the fragments which are lost are C_5H_9 and C_5H_8 respectively.

In view of the electron impact induced fragmentation of II it is interesting that upon catalytic hydrogenation, one mole of hydrogen is rapidly absorbed inducing the hydrogenolysis of a C_5H_9 fragment, to yield IVa, $C_{14}H_{15}O_2N$, showing an UV absorption spectrum very similar to II. In contrast to the latter, IVa yielded readily a methyl ether (IVb) upon treatment with diazomethane.[7]

Since IVa is not enough soluble in chloroform, its NMR spectrum was recorded in pyridine and DMSO solutions. In comparison with II, the pyridine solution spectrum showed major changes in the high and medium field regions, while the spectrum in DMSO clearly indicated that no changes had occurred in the aromatic protons region. The experimental difficulties in the analysis of the whole NMR spectrum of IVa were overcome by recording the spectrum of the methyl ether (IVb; *d*-chloroform solution).

Two singlets at δ 1·73 and 1·88 accounted for two vinylic Me groups; a two proton doublet at δ 3·48 was indicative for a methylene group split by one neighboring proton; the two peaks of the doublet were broadened by long range coupling, probably due to the Me protons. The sharp three proton singlet at δ 4·03 was assigned to the OMe group and the one proton multiplet at δ 5·3 was due to a vinylic hydrogen. The resonance peaks of the four aromatic protons showed the same pattern as in compounds I, II and III.

Comparison of the NMR spectra of compounds II and IV shows that the protons of the C_5H_9 fragment, lost during the conversion of the former to the latter, are those of two vinylic Me groups (singlets), of the more deshielded methylene (δ 4·57, doublet) and of a vinylic proton (multiplet). The reaction which took place (II → IV) can therefore be easily interpreted as the hydrogenolysis of an allylic ether, and according to the NMR data, the cleaved fragment (R_1 in B) can only be a 3,3-dimethylallyl chain. Accordingly, the formation of ions *m/e* 228 and 229 in the mass spectrum of II can be formulated as fission *a* (see formula C) of the allylic C—O bond for the former and elimination of a diene molecule (C_5H_8) for the latter case. By subtraction, the R_2 moiety in partial formula C must be also a C_5H_9 unit attached at C_3 and again, only a 3,3-dimethylallyl structure is compatible with the NMR features of IV as well as of II. Compound IVa is therefore 3-dimethylallyl-4-hydroxy-2-quinolone. Its mass spectrum is almost identical with that of dihydroflindersine (III), the main difference being the relative intensities of ions *m/e* 174 (base peak of III, but 92·2% of base in IVa) and *m/e* 186 (base peak in IVa *vs* 83·7% of base in III). In view of the structure of IVa, this can be explained by a very ready cyclization of its molecular ion to give III. Such a cyclization was actually achieved preparatively by Bowman and Grundon[2] who have obtained a mixture of dihydroflindersine and khaplofoline by treatment of IV with acid. Compound IVa has been prepared by Grundon[2] from aniline and diethyl 3-methyl-but-2-enyl-malonate and its reported physical constants are the same as those of the compound obtained by hydrogenolysis of II. Repetition of the acid

induced cyclization of IV (obtained from II) yielded 80% dihydroflindersine (III; identified by comparison with an authentic sample) and 20% of a second cyclization product to which we assign the structure of khaplofoline (IX) according to its physical properties (no direct comparison, however, was carried out).

This reaction corroborates the structure of IV, and thereby the structure of the naturally occurring compound II is established as 3-dimethylallyl-4-dimethylallyloxy-2-quinolone.

Some other degradation studies of II are described in the sequel, mainly in order to discuss the NMR spectra of the corresponding products. Upon catalytic hydrogenation of IVa, one mole of hydrogen is consumed yielding compound V in which the isopropylidene group is converted into an isopropyl group. This transformation was illustrated in the NMR spectrum of V by disappearance of the multiplet due to the vinylic proton in IV and the upfield shift of the signals of the two Me groups (doublet, δ 0·88).

Compound V still contains the Δ^3 benzylic double bond which could be cleaved with ozone. After esterification with diazomethane, two methyl esters VI and VII could be separated by chromatography.

The former (VI), containing the α-amido-ketone system, mol wt 277, (by mass spectrometry) is formed by simple cleavage of the ozonide, while the latter (VII), mol wt 279, is obtained by the subsequent reduction of the α-amido-ketone system to an α-amido-alcohol during the zinc–acetic acid work up of the crude ozonide. Indeed, when VI was exposed to zinc in acetic acid solution, it could be partly converted into VII.

The structures assigned to VI and VII are supported by their NMR spectra. The methylenic protons next to the ketone in VI give rise to a triplet at δ 3·00, while in VII no signals are present in this region of the spectrum; instead, two signals appear for the C<u>H</u>O<u>H</u> protons: the hydroxylic proton as a doublet at δ 3·17 (disappearing upon addition of D_2O) and the C<u>H</u>OH proton as a triplet at δ 4·25. The resonance signals of the aromatic protons of both VI and VII are almost superimposable, each of the four protons being well separated: in VI, double doublets at δ 8·10 and 8·75, accounting for the 5-H and 8-H respectively* and double triplets at δ 7·20 and 7·65, for the 6-H and 7-H respectively. In VII the corresponding signals appear at δ 8·06, 8·70, 7·10 and 7·55 respectively.

The deshielding of the 8-H in these compounds is due to the amidic oxygen and is indicative for its *cis* relation to the aromatic ring (restricted rotation around the amidic N-C bond). Such an effect has already been observed[8] in N-acyl indolines and N-acyl hexahydrocarbazoles in which the amidic CO is preferentially oriented towards the Ph ring. While in these cases, the NMR spectra contain also signals arising from the *trans* isomers, our compounds (VI and VII) seem to exist practically only in the *cis* conformation. The same situation has been observed in acetanilide[9] while N-methylacetanilide exists almost exclusively as the *trans* stereoisomer. For comparison, the NMR spectrum of N-acetyl methylanthranilate was recorded. The signals of the aromatic protons were practically superimposable on those of compounds VI and VII, hence it also exists in only one conformation with the acetamido oxygen *cis* to the aromatic ring.

* For convenience, the same numbering as for the quinolones has been used.

In contrast, in compound VIII obtained by ozonolysis of III, the acetamido group as part of a ring, is fixed in a conformation holding the amidic oxygen *trans* to the aromatic ring. As expected, the 8-H is no more deshielded, its signal being included in the complex multiplet at δ 7·10–7·70, together with the signals of 6-H and 7-H; the only deshielded aromatic proton in VIII is the 5-H giving rise to a double doublet centered at δ 8·00.

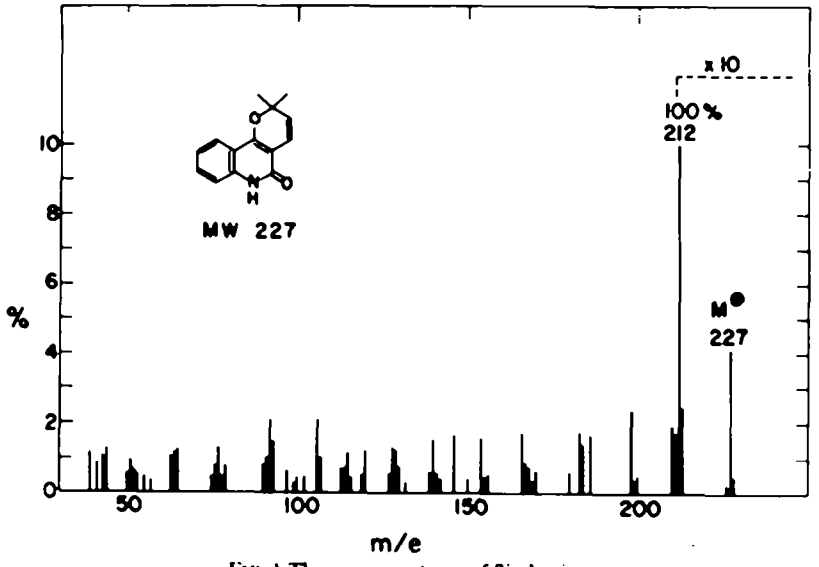


FIG. 1 The mass spectrum of flindersine

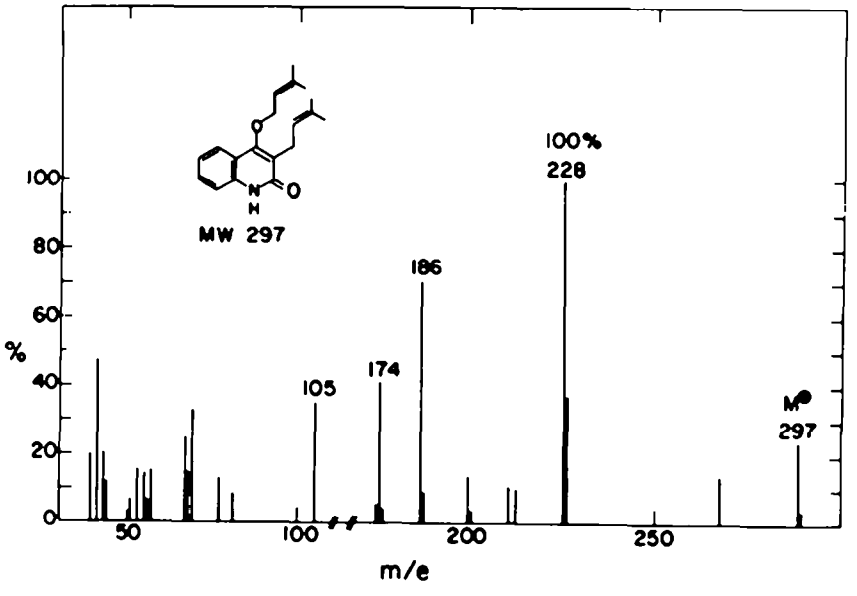


FIG. 2 The mass spectrum of 3-dimethylallyl-4-dimethylallyloxy-2-quinolone

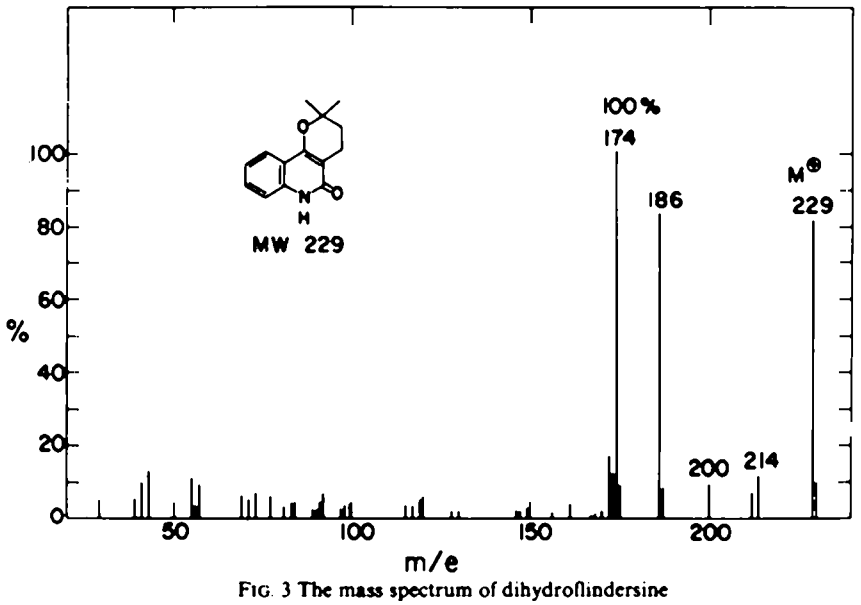


FIG. 3 The mass spectrum of dihydroflindersine

A compound related to II is 3-dimethylallyl-4-methoxy-2-quinolone, recently isolated from *Fagara zanthoxyloides* (Rutaceae).[10]

Many natural compounds contain one or more C_5 units in the molecule, which may be linked to a nuclear C atom of the main skeleton or to an O atom, forming an ether.[11] Addition of a C_5 unit to a 2,4-dihydroxy-quinoline intermediate and further cyclization, may lead to flindersine and khaplofoline. The cyclization step can be however precluded by etherification of the 4-OH leading in this case to a compound such as 3-dimethylallyl-4-dimethylallyloxy-2-quinolone (II), or to a 4-OMe analog such as the compound isolated[10] from *Fagara zanthoxyloides*.

EXPERIMENTAL

M.ps were taken on a Fisher–Johns apparatus and are uncorrected. IR spectra were recorded on a Perkin–Elmer Infracord model 137 spectrophotometer equipped with a NaCl prism; UV spectra were recorded on a Cary 14 instrument; NMR spectra were determined on a Varian A-60 spectrometer, for 5–10% solns in $CDCl_3$, d_6-DMSO or pyridine containing TMS as internal standard. TLC was done on chromatoplates of silica gel G (Merck) and spots were developed with I_2 vapors. Mass spectra were taken with an Atlas CH4 instrument, the samples being introduced directly into the source through a vacuum-lock; electron energy 70 eV, electron current 20 μA, source temp < 100°; secondary electron multiplier as the detector. Analyses were performed in the microanalytical laboratory of our Institute, under the direction of Mr. R. Heller.

Ground dry leaves and stems (2 kg) of *Haplophyllum tuberculatum* were extracted (soxhlet) with hexane for 24 hr. The hexane soln was concentrated to a volume of ~200 ml and adsorbed on a column of alumina (Alcoa F_{20}; 1·2 Kg). Elution with hexane-$CHCl_3$ (9:1) yielded fractions showing two spots on a chromatoplate (red coloration with Dragendorff reagent). These fractions (6 gr) were combined and rechromatographed through neutral alumina (Woelm, activity III). The product giving the upper spot was eluted with hexane-$CHCl_3$ (1:1) and after repeated crystallization from acetone yielded 1·3 gr. of II, m.p. 114–115°; ν_{max} 1603 and 1634 cm^{-1}; λ_{max} 227, 272, 324 and 338 mμ (ε 41,300, 32,200, 8500 and 5800). (Found: C, 76·73; H, 7·70; N, 4·66; M⁺, 297; $C_{19}H_{23}O_2N$ requires: C, 76·73; H, 7·80; N, 4·71%; M. wt. 297·38).

Elution with hexane–$CHCl_3$ (1:4) gave I showing the lower spot on the chromatoplate (2·8 g), m.p. 196–198° (from acetone). It was identified by direct comparison with an authentic sample.*

Upon catalytic hydrogenation in EtOH soln, over 10% Pd-C, at room temp and atm press, one mole H_2 was rapidly absorbed, yielding III m.p. 235–236° (from acetone–hexane), identical with a sample of this compound, obtained by synthesis.†[2]

Hydrogenolysis of II *to* IVa. Compound II (500 mg) in EtOH soln (100 ml) was hydrogenated over 10% Pd-C at room temp and atm press. After the absorption of one mole H_2 (~15 min) the reaction was discontinued, the catalyst was filtered off and the solvent removed in vacuum. The residue, in $CHCl_3$ solution, was filtered through a column of silica gel. The crystalline material which was obtained showed one spot on a chromatoplate and crystallized from acetone–hexane (300 mg) m.p. 175–177°;‡ ν_{max} 1650 cm^{-1}, λ_{max} 223, 262, 270 mμ (ε 40,000, 30,800, 30,700) and shoulders at 302 and 315 mμ. (Found: C, 73·67; H, 6·47; N, 6·20; M^+, 229; $C_{14}H_{15}O_2N$ requires: C, 73·34; H, 6·59; N, 6·11%; M. wt., 229·27).

Etherification with diazomethane in MeOH soln as described[7] afforded IVb, m.p. 132–133° (from acetone hexane); reported m.p. 132–134°[7] and 133°.[10]

Hydrogenation of IVa *to* V. Compound IVa (500 mg) was hydrogenated in EtOH soln as described above. One mole H_2 was taken up in about 2 hr, when the absorption ceased. The crude product, in $CHCl_3$ soln, was filtered through a silica gel column and then crystallized from $CHCl_3$, m.p. 158–160°; ν_{max} 1637 cm^{-1}; λ_{max} 222, 262 mμ (ε 44,000 and 28,000) and shoulders at 303 and 320 mμ. (Found: C, 72·72; H, 7·42; N, 6·00; M^+, 231; $C_{14}H_{17}O_2N$ requires: C, 72·70; H, 7·41; N, 6·06%; M. wt., 231·28%).

Ozonolysis of IVa. Compound IVa (400 mg) in EtOAc soln (70 ml) was ozonized at −15° for 10 min (~1·3 equivs of O_3). Most of the solvent was then removed in vacuum, AcOH (6 ml) and Zn powder (200 mg) were added and the mixture stirred for 2 hr at room temp. After filtration, the product was extracted with $CHCl_3$, the soln washed with water and then reextracted with a 10% Na_2CO_3 aq. The alkaline soln was acidified to pH ~2 (with HCl), the ppt was extracted with $CHCl_3$, dried over Na_2SO_4 and evaporated. The residue (200 mg) was esterified with diazomethane in ether soln, at room temp, for 30 min yielding a mixture of two esters which were separated by chromatography on neutral alumina (Woelm, activity II). Elution with hexane–$CHCl_3$ (9:1) yielded VI, 60 mg, m.p. 53–55° (from acetone–hexane) ν_{max}^{KBr} 1587, 1675, 1695 and 1721 cm^{-1}. (Found: C, 65·15; H, 6·80; N, 4·95; M^+, 277; $C_{15}H_{19}O_4N$ requires: C, 64·96; H, 6·91; N, 5·05%; M. wt., 277·31).

Elution with hexane–$CHCl_3$ (8:2) gave VII 25 mg, m.p. 88–90° (from acetone–hexane). ν_{max} 1580, 1655 and 1692 cm^{-1}. (Found: C, 64·42; H, 7·28; N, 4·97; M^+, 279; $C_{15}H_{21}O_4N$ requires: C, 64·49; H, 7·58; N, 5·01%; M. wt., 279·33). In several runs the proportions of these two compounds were variable.

Ozonolysis of dihydroflindersine (III). Dihydroflindersine (III; 480 mg) in EtOH (50 ml) was ozonized as described above. Following reductive workup (AcOH and Zn) for 2 hr at room temp, the product was extracted with $CHCl_3$, the soln washed with $NaHCO_3$ aq and water, dried over $NaSO_4$ and the solvent removed in vacuum. The crude product was chromatographed over neutral alumina. Elution with hexane–$CHCl_3$ (1:1) yielded 250 mg of pure product which crystallized from acetone–hexane m.p. 197–198°; ν_{max} 1603, 1634, 1664 and 1709 cm^{-1}. (Found: C, 63·98; H, 5·71; N, 5·68; M^+, 261: $C_{14}H_{15}O_4N$ requires: C, 64·36; H, 5·79; N, 5·36%; M. wt., 261·27).

Reduction of VI *to* VII. Compound VI (50 mg) in AcOH soln (3 ml) was stirred at room temp with Zn powder (30 mg) for 2 hr. After filtration, the product was taken up in $CHCl_3$, the soln washed with $NaHCO_3$ aq and water, dried over Na_2SO_4 and the solvent removed in vacuum. The residue (40 mg) showed two spots on a chromatoplate for a mixture of unreacted VI and of VII. An NMR spectrum of the mixture indicated that the two components are present in a ratio of ~1:1.

N-Acetyl methylanthranilate. NMR spectrum (*d*-chloroform soln): 3-H, d.d., δ 8·72; 4-H and 5-H d. tr. each δ 7·05 and δ 7·55; 6-H, d.d., δ 7·95; amidic proton, multiplet δ 11·09; $COOC\underline{H}_3$, δ 3·92; $NHCOC\underline{H}_3$, δ 2·23.

Acknowledgements—This work was supported in part by the National Council for Research and Development, Israel.

* Although the reported[1a] m.p. is 185–186° (crystallization from MeOH) the m.p. of the authentic sample of flindersine taken on the Fisher–Johns apparatus is also 196–198°; mixture m.p. was undepressed.

† Reported[2] m.p. 233–234° (from EtOAc). Dihydroflindersine obtained by the hydrogenation of flindersine[1] has m.p. 229° (also from EtOAc).

‡ Reported[2] m.p. for this compound obtained by synthesis is 180–182° (from aq. EtOH).

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