575. *Taxine. Part I. Isolation Studies and the Functional Groups of O-Cinnamoyltaxicin-I.*

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Taxine is shown to be a mixture of alkaloids (taxines). The major taxines are constructed from nitrogen-free polyhydroxylic compounds, here called taxicins, by partial esterification with 1 mol. of β-dimethylamino-β-phenylpropionic acid, and also with 1 or more mol. of acetic acid. Elimination of dimethylamine from the mixed taxines and acetylation of the non-basic product yields a mixture of acetylated *O*-cinnamoyltaxicins, of which three have been isolated crystalline.

The functional groups of *O*-cinnamoyltaxicin-I, which is derived from the major yew alkaloid, taxine-I, are shown in the expression (II; R = H).

The yew, *Taxus baccata* L., is one of the major poisonous plants of Europe; records of human fatalities due to its ingestion go back to classical times,[1] and fatalities among domestic animals due to yew poisoning are not uncommon today. About a century ago, Lucas [2] extracted from the leaves an ill-defined alkaloidal substance, to which the name taxine is usually given, and to which the poisonous properties are undoubtedly due. Until very recently, no crystalline or homogeneous alkaloidal derivatives had been obtained from taxine, its homogeneity was suspect, and it is therefore not surprising that little reliable is known of its chemical nature, despite numerous studies.[3] Indeed, of the earlier work, only that due to Winterstein and his colleagues,[4] summarised below, is significant. Our own studies on taxine, commenced in 1952, were therefore first aimed at isolating pure materials suitable for structural work. In a preliminary communication [5] we reported the isolation of *O*-cinnamoyltaxicin-I, a crystalline non-basic compound which is related in a known and simple manner to taxine-I, the main component of crude taxine, and a study of its functional groups. The present paper describes this work in detail.

Winterstein and his associates [4] obtained taxine as an amorphous (and therefore heterogeneous) substance, $[\alpha]_D$ +51·5°,* $C_{37}H_{51}NO_{10}$; it contained *ca.* 3—4 acetylatable hydroxyl groups. Acid hydrolysis gave acetic acid (*ca.* 1 mol.) and 1 mol. of a nitrogenous acid (Winterstein's acid). This was decomposed by heat into dimethylamine and cinnamic acid, which identified it as β-dimethylamino-β-phenylpropionic acid, a structure later confirmed by synthesis.[6] Taxine thus appeared to be an ester alkaloid, in which a nitrogen-free polyhydroxylic compound is partially esterified by 1 mol. of Winterstein's β-amino-acid and also by 1 or more mol. of acetic acid. The present results confirm this picture as applying to the main components of taxine, which is a mixture of bases. We propose to denote the component bases as taxine-I (the major alkaloid), etc., and to use the term taxicin (taxicin-I, etc.) to represent the acyl-free polyols from which the taxines are constructed by partial esterification as noted above.

At the outset of our experiments we found, in agreement with previous workers, that taxine is unstable in solution and also, to a smaller extent, in the dry state. The main, though not the only, cause of this instability is the β-amino-ester structure of the component taxines, which promotes the elimination of dimethylamine, giving the corresponding

* Optical rotations relate to solutions in chloroform, unless otherwise stated.

[1] Caesar, " The Gallic Wars," Book VI; see Everyman's Library, No. 702, Dent and Sons, Ltd., London, 1953, p. 109.

[2] Lucas, *Arch. Pharm.*, 1856, **95**, 145.

[3] For reviews, see Henry, " The Plant Alkaloids," 4th edn., Churchill Ltd., London, 1949; Graf and Bertholdt, *Pharm. Zentralhalle*, 1957, **96**, 385.

[4] Winterstein and Iatrides, *Z. physiol. Chem.*, 1921, **117**, 240; Winterstein and Guyer, *ibid.*, 1923, **128**, 175.

[5] Baxter, Lythgoe, Scales, Trippett, and Blount, *Proc. Chem. Soc.*, 1958, 9.

[6] Graf and Boedekker, *Arch. Pharm.*, 1956, **289**, 364.

cinnamates. We consequently did not pursue the isolation of the basic taxines, but preferred instead deliberately to complete the elimination of dimethylamine and to isolate the more stable cinnamate esters. A very mild and suitable elimination method had already been found by Dr. B. K. Blount, who most kindly informed us of his unpublished results, and this forms the foundation of our isolation method. When cold solutions of amorphous " taxine methiodide " and aqueous potassium carbonate are mixed, trimethylamine is set free from those components of taxine (and only those) which are esters of Winterstein's β-amino-acid, and an amorphous nitrogen-free precipitate, termed " desdimethylaminotaxine " by Blount, and composed of cinnamates, is formed. Our crude taxine preparations lost *ca.* 90% of their total nitrogen content as trimethylamine when submitted to Blount's procedure, so they were clearly composed very largely of esters of Winterstein's acid. In typical experiments, 10 kg. of dried yew clippings furnished 40 g. of crude taxine, which gave 28 g. of " desdimethylaminotaxine."

We were unable to separate crystalline materials directly from " desdimethylaminotaxine," but the products obtained from it by either mild acetylation or mild deacetylation were more tractable. Acetylation with cold acetic anhydride in pyridine gave a partially crystalline product; chromatography of the crystalline fraction on alumina gave as the major product an *O*-cinnamoyltaxicin-I triacetate, m. p. 237—239°, $[\alpha]_D$ +218°, together with smaller amounts of an *O*-cinnamoyltaxicin-II triacetate, m. p. 265—267°, $[\alpha]_D$ +137°. Similar chromatography of the syrupy fraction gave more of these two triacetates, together with small amounts of a third pure acetate cinnamate (see Experimental part), and the progress of the separation showed the presence of still further similar compounds, not as yet obtained crystalline. Thus the nature of taxine as a complex of esters of Winterstein's acid was established.

An alternative isolation procedure, which we have found suitable for routine work, was to treat " desdimethylaminotaxine " with cold, very dilute methanolic sodium methoxide (Zemplén methanolysis [7]), which removed acetate groups without affecting the cinnamate groups. The main component of the product, *O*-cinnamoyltaxicin-I, was then separable by direct crystallisation, although it could not be completely freed in this way from traces of a taxicin-II derivative. However, since the two series of compounds were usually readily separable at a later stage, the very slightly impure material was acceptable for most purposes. (Rigorously pure *O*-cinnamoyltaxicin-I, m. p. 233—234°, $[\alpha]_D$ +285°, was best obtained by Zemplén methanolysis of its triacetate, $[\alpha]_D$ +218°, described above, but the process was rather wasteful.) It was profitable to re-acetylate the syrupy material contained in the mother-liquor from which the crude *O*-cinnamoyltaxicin-I had separated, and to chromatograph the product in order to recover from it the two triacetates, $[\alpha]_D$ +218° and +137°, already mentioned. In this way, 28 g. of " desdimethylaminotaxine " furnished 7·7 g. of *O*-cinnamoyltaxicin-I together with 4·4 g. of its triacetate, and 1·9 g. of *O*-cinnamoyltaxicin-II triacetate.

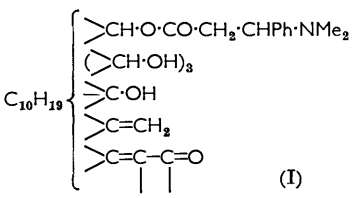
In the following paragraphs we describe chemical studies on *O*-cinnamoyltaxicin-I which largely define its functional groups as in the expression (II; R = H). This leads to the expression (I) for deacetyltaxine-I; since our isolation procedures involved addition or removal of acetate groups the number of the latter present in native taxine-I remains for the present undefined. In working with derivatives of taxicin-I and -II it is useful to note the following. In general they are characterised by low powers of crystallisation and instability towards acids and alkalis. Many require storage at −40° for their proper preservation; for many (not all), optical rotations are more characteristic than m. p.s. Some form solvates, particularly with benzene, which may give rise to analytical difficulties.

The analytical data on *O*-cinnamoyltaxicin-I and on a series of its derivatives establish its formula as $C_{29}H_{36}O_7$. Taxicin-I, $C_{20}H_{30}O_6$, from which it is derived, has not so far

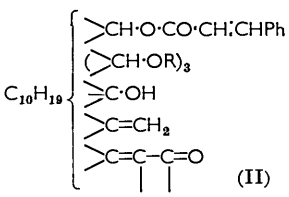
[7] Zemplén and Kuns, *Ber.*, 1923, **56**, 1705.

been isolated; hydrolysis of the cinnamate with cold dilute aqueous alkali liberated cinnamic acid, but further degraded the taxicin-I residue. *O*-Cinnamoyltaxicin-I is a neutral compound, unaffected by brief treatment with diazomethane. The cinnamate double bond was the site of the controlled reduction with palladium and 1 mol. of hydrogen, which gave *O*-β-phenylpropionyltaxicin-I (III; R = H). This furnished β-phenylpropionic acid on alkaline hydrolysis; here, too, the taxicin-I residue was further degraded. The β-phenylpropionate (III; R = H) afforded the first opportunity for a study of the ultraviolet absorption of the taxicin-I residue; it showed a maximum near 280 mμ (ε 5700) which in the cinnamate (II; R = H) was masked by the more intense cinnamate absorption near the same wavelength. The nature of the chromophore causing this absorption is briefly discussed below. A similar absorption, varying somewhat in position (278 ± 5 mμ) and intensity, was displayed by all the taxicin-I derivatives described in this paper. Associated with this absorption was an infrared band near 1675 cm.$^{-1}$, which in the cinnamate (II; R = H) and the phenylpropionate (III; R = H) accompanied the expected ester bands in the 6 μ region; this 1675 cm.$^{-1}$ band, too, was shown by all the taxicin-I derivatives here described. It was clearly due to a conjugated carbonyl group forming part, possibly, of an acylic system, but more probably of an unstrained ring.

O-Cinnamoyltaxicin-I contains three readily acetylatable hydroxyl groups. Reaction with an excess of acetic anhydride in pyridine gave the triacetate (II; R = Ac), $[\alpha]_D$ +218°, mentioned above. Acetylation of the β-phenylpropionate (III; R = H) also gave a triacetate (III; R = Ac). This compound was also obtained by hydrogenation of the triacetate (II; R = Ac) with palladium; the reaction stopped cleanly when 1 mol. of hydrogen had been taken up. Zemplén methanolysis of the triacetates (II and III; R = Ac) gave back their acetate-free precursors; the arylacyl groups were not removed by this treatment, and it seemed probable that this was due to the situation or stereochemistry of the hydroxyl group which these residues esterify. The three reactive hydroxyl groups in the cinnamate (II; R = H) were inert to *p*-nitrobenzoyl chloride in pyridine at room temperature; in view of their ready acetylation, this was surprising; presumably it is due to steric factors. The reactive hydroxyl groups are regarded as secondary.



(I)



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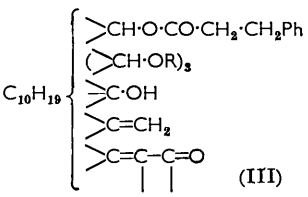

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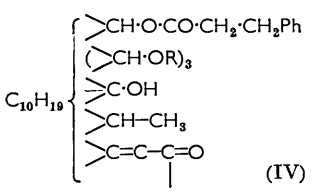
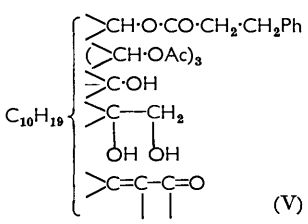


(IV)



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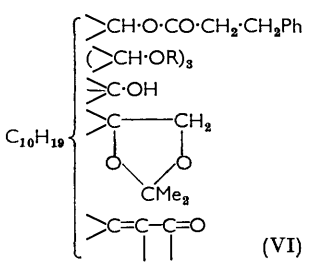

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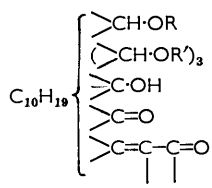

(VII)

The triacetates (II and III; R = Ac) contained a free hydroxyl group, the presence of which was indicated by their adsorption affinity for alumina. Both showed the expected O–H absorption near 3560 cm.$^{-1}$. When the triacetate (III; R = Ac) was dissolved in ethyl deuteroxide and then re-isolated, this band was abolished and in its place appeared strong O–D absorption near 2618 cm.$^{-1}$. Some other derivatives which contained this free hydroxyl group were unaffected by mild treatment with chromic acid, so it is probable that it is a tertiary hydroxyl group. This defines provisionally the nature of all the seven oxygen atoms of *O*-cinnamoyltaxicin-I.

In addition to the conjugated unsaturated system of taxicin-I to which the ultraviolet absorption near 280 mμ is due, an isolated and reactive double bond is present. Thus *O*-β-phenylpropionyltaxicin-I (III; R = H), unlike its triacetate (III; R = Ac), was further reduced by hydrogen and palladium, giving dihydrotaxicin-I β-phenylpropionate (IV; R = H). This formed a triacetate (IV; R = Ac) from which it was regenerated by Zemplén methanolysis. The spectral data on these two compounds showed that they contained the original taxicin-I chromophore.

The nature of the isolated double bond was determined by hydroxylation and glycol fission. *O*-β-Phenylpropionyltaxicin-I triacetate (III; R = Ac) was converted into a vicinal glycol (V) either by neutral permanganate in acetone or by the use of osmium tetroxide in pyridine; it is of interest that these reagents did not attack the taxicin-I chromophore at any useful rate even when used in excess: the spectral properties of the glycol (V) showed that this chromophore remained intact. The glycol formed an isopropylidene derivative (VI; R = Ac) which was successfully deacetylated to the tetraol (VI; R = H); these two steps from the glycol (V) could be retraced by use of the usual reagents.

Lead tetra-acetate oxidised the glycol (V), giving formaldehyde and a dicarbonyl compound (VII; R = $CO{\cdot}CH_2{\cdot}CH_2Ph$, R′ = Ac) which formed only a monoxime. The dicarbonyl compound was inert to mild treatment with chromic acid. On treatment with cold dilute methanolic sodium methoxide it lost both the acetate and also the phenylpropionate group, giving oxonortaxicin-I * (VII; R = R′ = H) which readily formed a tetra-acetate. All these compounds contained the original taxicin-I chromophore. Oxonortaxicin-I showed the original conjugated carbonyl band near 1675 cm.$^{-1}$ and also a new band near 1710 cm.$^{-1}$, suggesting a keto-group in an acyclic system, or, more probably, in a six-membered ring. Neither carbonyl group was aldehydic, since the easily identified aldehydic proton signal was absent from the nuclear magnetic resonance spectrum † of the compound. These results show that the isolated double bond of taxicin-I is of the asymmetrically disubstituted type $>C{=}CH_2$.

We consider next the nature of the taxicin-I chromophore: it clearly contains an αβ-unsaturated ketone system; the question arises, is it confined to that system? No enone of which we are aware has its *K*-band above 260 mμ, and although the bathochromic effects of strain on such systems have lately been demonstrated,[8] an enone with its *K*-band near 280 mμ would be remarkable. Alternatives are (*a*) a conjugated dienone or (*b*) cyclopropane conjugation of the enone, as in cycloartenone,[9] which has its *K*-band near 269 mμ; so far no evidence for either of these alternatives has been obtained.‡ Determination of the number of double bonds in the taxicin-I chromophore was hindered by their chemical

* In this trivial name, nor denotes replacement of $:CH_2$ by H_2.

† Obtained on a solution in dioxan with a Varian model 4300B spectrometer by Mr. J. Feeney of the University of Liverpool, whom we thank most warmly.

‡ We have failed to substantiate observations on the reduction of the isomerised dihydrotaxicin-I β-phenylpropionate triacetate by sodium borohydride which were reported earlier in ref. 5.

[8] Büchi, Erickson, and Wakabayashi, *J. Amer. Chem. Soc.*, 1961, **83**, 927, and references there cited.

[9] Spring, Irvine, and Henry, *J.*, 1955, 1316; for another example of a similar chromophore, see Gnoj, Oliveto, Robinson, and Barton, *Proc. Chem. Soc.*, 1961, 207.

inertness. The keto-group in the cinnamate (II; R = H) failed to react with the usual ketonic reagents. The conjugated double bond was not readily attacked by oxidising agents, as remarked above, and was also difficult to hydrogenate. Thus with platinum in acetic acid, dihydrotaxicin-I β-phenylpropionate triacetate (IV; R = Ac) took up only 3 mol. of hydrogen, which saturated the phenyl group, giving dihydrotaxicin-Iβ -cyclohexylpropionate triacetate, in which the original chromophore was still present. Dihydrotaxicin-I β-phenylpropionate (IV; R = H) was slowly reduced by sodium borohydride in alcohol to a product which, although it appeared from thin-layer chromatography to be homogeneous, could not be obtained crystalline. It showed no intense maximum above 220 mμ and was therefore not a conjugated diene; the keto-group had been reduced, since the band near 1675 cm.$^{-1}$ was absent; unfortunately it was not possible to demonstrate whether or not a double bond had also been reduced. This result, although it favours the conclusion that the chromophore contains only one ethylenic link is therefore not decisive, and further work is in progress.

In conclusion, work on taxine from other laboratories must be summarised. Our isolation studies and almost all the above chemical work had been completed when notable isolation studies due to Graf and his colleagues were described in a lecture summary.[10] Taxine was shown to be a mixture of alkaloids, of which three were isolated crystalline as such or as derivatives, *viz.*: taxine A,[11] $C_{35}H_{49}NO_{10}$, m. p. 204—206°, $[\alpha]_D$ −140°; taxine B, the major alkaloid, $C_{35}H_{51}NO_9$, $[\alpha]_D$ +119°; and taxine C, m. p. 221°. After our preliminary publication,[5] further details of taxine B were published.[12] Its formula was corrected to $C_{33}H_{45}NO_8$, and was shown to contain one acetate and one β-dimethylamino-β-phenylpropionate group together with three hydroxyl groups, of which two were readily acetylatable. It showed $\lambda_{max.}$ *ca.* 280 mμ and $\nu_{max.}$ 1675 cm.$^{-1}$, which were ascribed to a conjugated enone system. From these results it seems likely that Graf's taxine B is identical with our taxine-I. Di-*O*-acetyltaxine B methiodide and aqueous potassium carbonate gave a triacetate cinnamate which should be identical with our *O*-cinnamoyltaxicin-I triacetate, but its constants were not recorded, and, with this possible exception, none of the actual compounds described by Graf were the same as those described in the present paper.

Japanese workers [13,14] have continued their studies on taxinin, a nitrogen-free compound obtained from *Taxus baccata* L. (subspecies *cuspidata*). The situation with regard to this compound is at present confused. It is said to have m. p. 266—267°, $[\alpha]_D$ +128·3°, and the formula $C_{30}H_{34}O_8$ [13] or $C_{31}H_{38-40}O_8$.[14] It has spectral characteristics resembling those of our taxicin-I compounds, *viz.*, $\nu_{max.}$ 1680 cm.$^{-1}$ and $\lambda_{max.}$ 275 mμ [a maximum at 345 mμ (log ε 2·33) is also recorded]. Hydrolysis evidence shows it to be an acetate cinnamate, and it is devoid of free hydroxyl groups.

Kondo and Taga [13] appear to regard our *O*-cinnamoyltaxicin-I triacetate as identical with their taxinin, but this seems to us most improbable. We think, however, that taxinin may well be identical with our *O*-cinnamoyltaxicin-II triacetate, m. p. 265—267°, $[\alpha]_D$ +137°. This compound contains no free hydroxyl groups, has the formula $C_{35}H_{42}O_9$, and shows spectral characteristics closely similar to those of the taxicin-I series. Hydrogenation with palladium converted it into *O*-β-phenylpropionyltaxicin-II triacetate, m. p. 239°, $[\alpha]_D$ +78°, and it is possible that the " tetrahydrotaxinin " of the Japanese workers, which was said to have m. p. 234—235°, $[\alpha]_D$ +44·93°, may be an impure form of this compound.

[10] Graf *et al.*, *Angew. Chem.*, 1956, **68**, 249.
[11] For further details see Graf and Bertholdt, *Pharm. Zentralhalle*, 1957, **96**, 385.
[12] Graf, *Arch. Pharm.*, 1958, **291**, 443.
[13] Kondo and Taga, *Ann. Rep. ITSUU Lab.*, 1958, **9**, 67, 71; Kondo, Taga, and M. Takahashi, *ibid.*, 1959, **10**, 43; Taga, *Chem. and Pharm. Bull.* (*Japan*), 1960, **8**, 934.
[14] T. Takahashi, Ueda, Oishi, and Minamoto, *Chem. and Pharm. Bull.* (*Japan*), 1958, **6**, 728; Takahashi, Ueda, Maki, and Minamoto, *ibid.*, 1960, **8**, 372.

Experimental

Ultraviolet data refer to solutions in alcohol.

Extraction of Taxine and Conversion into "Desdimethylaminotaxine" (with Dr. B. K. Blount).—Yew leaves (10 kg.) which had been dried in a current of air at 40° for 3 days were soaked in 0·65% v/v sulphuric acid (50 l.) with occasional stirring for 7 days; the extract was then separated from the leaves, which were then twice re-extracted in the same way. Each of the extracts was brought to pH 9 by addition of aqueous ammonia, and through the well-stirred extract a current of carbon tetrachloride was circulated by a pump; after passing through the basified extract it was passed through a long and narrow column (90 × 5 cm.) of 10% sulphuric acid, from the foot of which it was returned to the basified solution. After 8 hr. all the taxine had been transferred from the basified solution to the sulphuric acid solution, from which it was obtained by basification as before and ordinary extraction with ether. Evaporation of the washed and dried ethereal solution gave crude taxine (total 40 g.) as a colourless amorphous powder.

To a solution of crude taxine (20 g.) in ether (100 c.c.) methyl iodide (10 c.c.) was added, and after 24 hr. the pale yellow amorphous methiodide was collected, washed with ether, and dried. Its solution in alcohol (100 c.c.) was added with stirring during 90 min. to a solution of potassium carbonate (25 g.) in water (1·4 l.); stirring was continued for a further 2 hr., after which the precipitated "desdimethylaminotaxine" (14 g.) was collected, washed with water, and dried.

Isolation of the Triacetates of O-Cinnamoyltaxicin-I and O-Cinnamoyltaxicin-II.—"Desdimethylaminotaxine" (10 g.), pyridine (30 c.c.), and acetic anhydride (16 c.c.) were kept together for 3 days, the excess of reagent was decomposed with alcohol (12 c.c.), and after the solution had been diluted with benzene (250 c.c.) it was washed five times with dilute sulphuric acid and then with water, and dried. Evaporation gave a gum which crystallised from benzene in prisms (3·5 g.) containing benzene of crystallisation. The crude crystallisate was chromatographed from benzene on neutral alumina (grade II; 300 g.); development with benzene–alcohol (2000 : 1) eluted material which separated from alcohol as prisms (100 mg.) of *O-cinnamoyltaxicin-II triacetate*, m. p. 265—267°, $[\alpha]_D^{18}$ +137° (Found: C, 69·5; H, 6·85. $C_{35}H_{42}O_9$ requires C, 69·3; H, 7·0%), $\lambda_{max.}$ 279 mμ (ε 28,500) and $\nu_{max.}$ (in $CHCl_3$) 1735 (acetate C=O), 1704 (cinnamate C=O), 1675 (conjugated ketone), and 1645 (cinnamate C=C) cm.$^{-1}$ (no hydroxylic absorption).

Continued development with benzene–alcohol (500 : 1) eluted material which on recrystallisation from alcohol gave *O-cinnamoyltaxicin-I triacetate* (2·3 g.) as prisms m. p. 237—239°, $[\alpha]_D^{18}$ +218° (Found: C, 67·2; H, 6·55. $C_{35}H_{42}O_{10}$ requires C, 67·5; H, 6·8%), $\lambda_{max.}$ 281 mμ (ε 26,500), $\nu_{max.}$ (in $CHCl_3$) 3663 (OH), 1742 (acetate C=O), 1709 (cinnamate C=O), 1675 (conjugated ketone), and 1647 (cinnamate C=C) cm.$^{-1}$. Acetyl groups (determined by transesterification): 3·0.

Deacetylation of "Desdimethylaminotaxine."—A solution of "desdimethylaminotaxine" (20 g.) in absolute methanol (200 c.c.) was cooled to 0°, made 0·025N in sodium methoxide, and kept at 0° for 16 hr., after which it was acidified with glacial acetic acid and evaporated to dryness under reduced pressure. The residue was extracted with water and ether, and the ether extracts were washed, dried, and evaporated to 50 c.c. Benzene (100 c.c.) was then added, and the evaporation was continued slowly at atmospheric pressure to a small volume, which contained a crystalline mass. This was broken up, collected, washed with ether, and recrystallised from methanol–water. Recrystallisation from ethyl acetate–light petroleum (b. p. 60—80°) gave slightly impure *O*-cinnamoyltaxicin-I (5·5 g.) as needles m. p. 231—232°, $[\alpha]_D^{18}$ +281°. The residual material (13 g.) was acetylated as described above, furnishing crystalline material (3·4 g.) and an amorphous residue (11 g.). Chromatography of the crystalline material in the way described above gave *O*-cinnamoyltaxicin-II triacetate (200 mg.) and *O*-cinnamoyltaxicin-I triacetate (2·8 g.). Chromatography of the amorphous material in a similar manner gave, in the order of their elution, further *O*-cinnamoyltaxicin-II triacetate (1·2 g.), an acetylated *O*-cinnamoyltaxicin-III (70 mg.), m. p. 254—255°, $[\alpha]_D^{18} < \pm 1°$, and more *O*-cinnamoyltaxicin-I triacetate (0·4 g.).

O-Cinnamoyltaxicin-I.—Deacetylation of the triacetate in methanol–chloroform with methanolic sodium methoxide as described above gave pure *O-cinnamoyltaxicin-I*, m. p. 233—234°, $[\alpha]_D^{21}$ +285° (Found: C, 70·0; H, 7·3. $C_{29}H_{36}O_7$ requires C, 70·1; H, 7·3%), $\lambda_{max.}$ 282 mμ

(ε 28,200), $\nu_{max.}$ (in $CHCl_3$) 1703 (cinnamate C=O), 1671 (conjugated C=O), and 1645 (cinnamate C=C) cm.$^{-1}$. Acetylation with acetic anhydride in pyridine gave back the triacetate.

O-β-*Phenylpropionyltaxicin-I Triacetate.*—A solution of *O*-cinnamoyltaxicin-I triacetate (0·8 g.) in ethyl acetate (50 c.c.) containing 5% palladium–charcoal (200 mg.) was shaken with hydrogen (uptake, 1 mol.). Evaporation of the filtered solution and crystallisation of the residue from alcohol gave O-β-*phenylpropionyltaxicin-I triacetate* as needles (0·7 g.), m. p. 178—179°, $[\alpha]_D^{19}$ +138° (Found: C, 67·3; H, 7·1. $C_{35}H_{44}O_{10}$ requires C, 67·3; H, 7·1%), $\lambda_{max.}$ 276 mμ (ε 6400), $\nu_{max.}$ (in $CHCl_3$) 1724 (ester C=O), 1675 (conjugated C=O), and 3560 (–OH) cm.$^{-1}$.

O-β-*Phenylpropionyltaxicin-II Triacetate.*—Similar hydrogenation of *O*-cinnamoyltaxicin-II triacetate gave O-β-*phenylpropionyltaxicin-II triacetate* which separated from alcohol as needles, m. p. 239°, $[\alpha]_D^{18}$ +78° (Found: C, 69·2; H, 7·2. $C_{35}H_{44}O_9$ requires C, 69·05; H, 7·3%), $\lambda_{max.}$ 268 mμ (ε 7400), $\nu_{max.}$ (in $CHCl_3$) 1736 (ester C=O) and 1676 (conjugated C=O) cm.$^{-1}$, but no hydroxylic absorption in the 3 μ region.

O-β-*Phenylpropionyltaxicin-I.*—The corresponding triacetate (2 g.) was kept for 16 hr. at 0° with 0·025N-methanolic sodium methoxide, then acidified with glacial acetic acid. The product, isolated from the evaporated solution in the usual manner, crystallised from aqueous methanol, giving O-β-*phenylpropionyltaxicin-I* (0·95 g.), m. p. 206—207·5°, $[\alpha]_D^{18}$ +176° (Found: C, 69·7; H, 7·9. $C_{29}H_{38}O_7$ requires C, 69·85; H, 7·7%), $\lambda_{max.}$ 280 mμ (ε 5700), $\nu_{max.}$ (in CCl_3) 1724 (ester C=O) and 1675 (conjugated C=O) cm.$^{-1}$.

Hydrolysis with aqueous-alcoholic N-potassium hydroxide at room temperature gave β-phenylpropionic acid, isolated as the *S*-benzylisothiouronium salt, m. p. 153°. Acetylation of *O*-β-phenylpropionyltaxicin-I with acetic anhydride in pyridine gave back the triacetate, m. p. 178—179°.

Hydrogenation of O-*Cinnamoyltaxicin-I with Palladised Charcoal.*—(*a*) The cinnamate (3·0 g.), dissolved in ethyl acetate (150 c.c.) containing 5% palladised charcoal (1 g.), was shaken with hydrogen until the initial rapid uptake of 1 mol. was complete (20 min.). Filtration, evaporation, and crystallisation from aqueous methanol gave *O*-β-phenylpropionyltaxicin-I (2·5 g.), m. p. 206—207·5°, identical with that prepared as described above.

(*b*) When the above hydrogenation was continued till hydrogen uptake ceased (24 hr.; 2·15 mol. absorbed), the product crystallised from aqueous methanol, giving crude dihydrotaxicin-I β-phenylpropionate (1·9 g.), m. p. 205—206°. Acetylation with acetic anhydride and pyridine in the usual manner, and chromatographic purification on alumina (grade II) from benzene gave *dihydrotaxicin-I β-phenylpropionate triacetate* (1·4 g.) (from alcohol), m. p. 230—231·5°, $[\alpha]_D^{18}$ +160° (Found: C, 67·2; H, 7·4. $C_{35}H_{46}O_{10}$ requires C, 67·1; H, 7·4%), $\lambda_{max.}$ 276 mμ (ε 5000) and $\nu_{max.}$ (in $CHCl_3$) 1736 (ester C=O) and 1678 (conjugated C=O) cm.$^{-1}$.

Deacetylation of the triacetate by the Zemplén method afforded pure *dihydrotaxicin-I* β-*phenylpropionate* as needles (from aqueous methanol), m. p. 209—209·5°, $[\alpha]_D^{21}$ +217° (Found: C, 69·55; H, 7·85. $C_{29}H_{40}O_7$ requires C, 69·55; H, 8·05%), $\lambda_{max.}$ 283 mμ (ε 5200), $\nu_{max.}$ (in $CHCl_3$) 1724 (ester C=O) and 1675 (conjugated C=O) cm.$^{-1}$.

Hydroxylation of O-β-*Phenylpropionyltaxicin-I Triacetate.*—(*a*) The triacetate (1·25 g.) was dissolved in acetone (60 c.c.) and water (5 c.c.) and stirred with hydrated magnesium sulphate (4 g.) whilst a 1% solution of potassium permanganate in acetone (29 c.c.) was added during 75 min. The mixture was then warmed briefly to coagulate the manganese dioxide, the filtered solution was evaporated, and the residue was dissolved in chloroform (40 c.c.) and washed with water. Evaporation of the chloroform solution gave a solid, which crystallised from benzene giving the *glycol* (0·71 g.), m. p. 192—195°, $[\alpha]_D^{18}$ +167° (Found: C, 63·95; H, 7·0. $C_{35}H_{46}O_{12}$ requires C, 63·8; H, 7·05%), $\lambda_{max.}$ 275 mμ (ε 5800), $\nu_{max.}$ (in $CHCl_3$) 1675 cm.$^{-1}$.

(*b*) *O*-β-Phenylpropionyltaxicin-I triacetate (200 mg.) and osmium tetroxide (200 mg.) were kept together for 16 hr. in ether (15 c.c.) and pyridine (0·3 c.c.). The crystalline osmate was collected, washed with dry ether, and dissolved in pyridine (3 c.c.). The solution was stirred with sodium hydrogen sulphite (360 mg.) in water (6 c.c.) and pyridine (5 c.c.) for 2 hr., after which it was extracted with chloroform (3 × 10 c.c.), and the extracts were united and washed thoroughly with dilute hydrochloric acid and then with water. Evaporation of the solvent and crystallisation from benzene gave the glycol (165 mg.), m. p. 204—205°, $[\alpha]_D^{19}$ +168°.

A portion (106 mg.) of the glycol, dry acetone (25 c.c.), and anhydrous copper sulphate (2 g.) were shaken together for 3 days; the solution was then filtered and the filtrate was shaken for 30 min. with potassium carbonate (1 g.). Filtration and evaporation of the solvent gave an oil which crystallised from ethyl acetate–light petroleum (b. p. 60—80°), giving the

isopropylidene derivative (90 mg.) as needles m. p. 218—219°, $[\alpha]_D^{20}$ +163° (Found: C, 65·5; H, 6·85. $C_{38}H_{50}O_{12}$ requires C, 65·3; H, 7·2%), $\lambda_{max.}$ 276 mμ (ε 4900) and $\nu_{max.}$ (in $CHCl_3$) 1678 cm.$^{-1}$. Hydrolysis with hot 50% acetic acid for 30 min. regenerated the glycol.

Zemplén deacetylation of the isopropylidene derivative (210 mg.) in the normal manner gave a *tetraol* (100 mg.) as prisms (from benzene–hexane), m. p. 196°, $[\alpha]_D^{20}$ +202° (Found: C, 67·15; H, 7·6. $C_{32}H_{44}O_9$ requires C, 67·1; H, 7·75%), $\lambda_{max.}$ 282 mμ (ε 5300). Acetylation in the usual manner gave back the triacetate.

Oxonortaxicin-I.—The above glycol of $[\alpha]_D$ +168° (0·64 g.) and lead tetra-acetate in chloroform (19 c.c.; 0·054M) were kept together for 30 min. at room temperature; quantitative determinations had shown that 1·03 mol. of the oxidant were reduced. The solution was then diluted with chloroform (30 c.c.), washed well with water, dried, and evaporated. The *oxonortaxicin-I β-phenylpropionate triacetate* (0·5 g.) separated from ethyl acetate–light petroleum (b. p. 60—80°) as needles, m. p. 198—199°, α_D^{18} +133°, $\lambda_{max.}$ 273 mμ (ε 6400), $\nu_{max.}$ (in $CHCl_3$) 1675 cm.$^{-1}$ (Found: C, 65·1; H, 6·7. $C_{34}H_{42}O_{11}$ requires C, 65·2; H, 6·75%). The *oxime*, obtained by refluxing the ketone with hydroxylamine hydrochloride in pyridine and alcohol, had m. p. 227° (Found: C, 63·5; H, 6·5; N, 2·25. $C_{34}H_{43}NO_{11}$ requires C, 63·5; H, 6·75; N, 2·2%). The aqueous washings from the preparation of the above triacetate contained formaldehyde, isolated in 83% yield as the dimedone derivative, m. p. 189—190°.

Treatment of the above triacetate (900 mg.) with 0·026N-methanolic sodium methoxide (27 c.c.) at 0° for 24 hr., followed by isolation in the usual manner, gave *oxonortaxicin-I* as prisms (325 mg.) (from ether), m. p. 225—227° (decomp.), $[\alpha]_D^{19}$ +229° (in alcohol) (Found: C, 61·6; H, 7·55. $C_{19}H_{28}O_7$ requires C, 61·9; H, 7·65%), $\lambda_{max.}$ 278 mμ (ε 5400), $\nu_{max.}$ (in Nujol) 1672 and 1712 cm.$^{-1}$. The *oxime* formed needles (from ether), m. p. 296° (decomp.) (Found: N, 3·95. $C_{19}H_{29}NO_7$ requires N, 3·75%). The *tetra-acetate* crystallised from ethyl acetate–hexane as needles, m. p. 216—217°, $[\alpha]_D^{16}$ +163° (Found: C, 60·3; H, 6·85. $C_{27}H_{36}O_{11}$ requires C, 60·45; H, 6·75%), $\lambda_{max.}$ 273 mμ (ε 5750).

Catalytic Reduction of Dihydrotaxicin-I β-Phenylpropionate Triacetate.—The triacetate (104 mg.) in glacial acetic acid (10 c.c.) containing Adams platinum catalyst (25 mg.) was shaken with hydrogen at room temperature. In 2 hr. 3·03 mol. were taken up, after which reaction ceased. The product, isolated in the usual way, separated from hexane as needles (80 mg.) of *dihydrotaxicin-I β-cyclohexylpropionate triacetate*, m. p. 204—205°, $[\alpha]_D^{18}$ +168° (Found: C, 66·3; H, 8·25. $C_{35}H_{52}O_{10}$ requires C, 66·4; H, 8·3%), $\lambda_{max.}$ 276 mμ (ε 5700), $\nu_{max.}$ (in $CHCl_3$) 1740 and 1675 cm.$^{-1}$. Benzenoid absorption was absent from the spectrum of Nujol suspension.

Reduction of Dihydrotaxicin-I β-Phenylpropionate with Sodium Borohydride.—The β-phenylpropionate (200 mg.) and sodium borohydride (20 mg.) were kept together in alcohol (6 c.c.) containing ethyl acetate (3 c.c.) for 2½ hr.; preliminary tests showed that the reaction required this time. The solution was acidified with acetic acid, then diluted with water, and the product was isolated with ether in the usual way. It formed a gum (190 mg.) which gave only one spot on thin-layer chromatography. It showed no high-intensity absorption above 220 mμ, and no band near 1675 cm.$^{-1}$.

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