Vetispirane Sesquiterpene Glucosides from Flue-cured Virginia Tobacco: Structure, Absolute Stereochemistry, and Synthesis. *X*-Ray Structure of the *p*-Bromobenzenesulphonate of One of the Derived Aglycones

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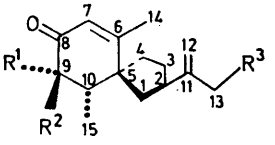
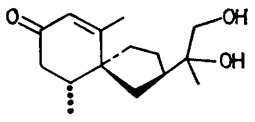
Summary The structure and absolute stereochemistry of four new vetispirane sesquiterpene glucosides from flue-cured Virginia tobacco have been deduced and three of them have been synthesised from (−)-solavetivone; the *X*-ray structure of the *p*-bromobenzenesulphonate of one of the derived aglycones is reported.

THE isolation, structural elucidation, and flavourant characteristics of the constituents of different varieties of tobacco leaf is an area of active research.[1] Our interest in this field has prompted us to carry out a sequential fractionation of the water-soluble portion of an acetone extract of flue-cured Virginia tobacco by ion exchange, gel permeation, and preparative high pressure liquid chromatography



(1) $R^1=R^2=R^3=H$
(2) $R^1=R^3=H$, $R^2=OH$
(3) $R^1=R^2=H$, $R^3=OH$
(4) $R^1=OH$, $R^2=R^3=H$
(5) $R^1=R^3=H$, $R^2=p$-$BrC_6H_4SO_3$



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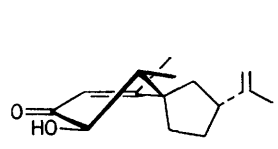

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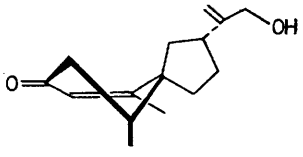

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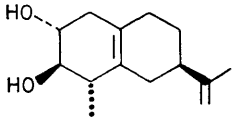

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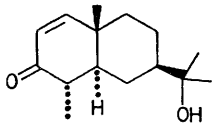
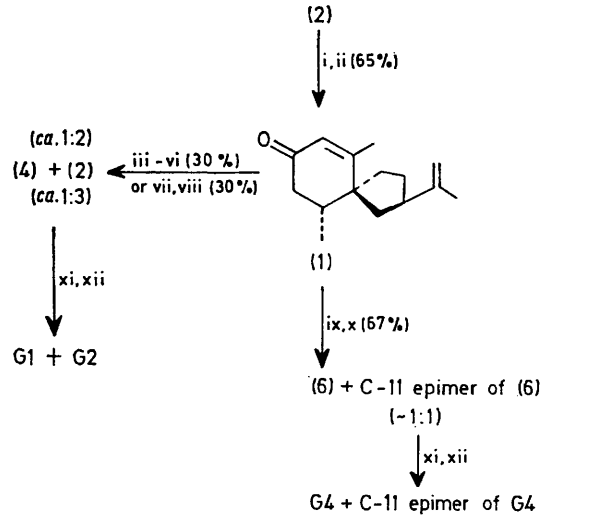


(10)

(h.p.l.c.). This technique has resulted in the isolation of four glucosides, G1—4, in amounts totalling approximately 70 p.p.m. of the flue-cured leaf. A feature of these glucosides is the genuine tobacco aroma which they impart on pyrolysis.[2]

A combination of ^1H and ^{13}C n.m.r. spectroscopy and enzymatic hydrolysis with β-glucosidase (except in the case of G1 which was practically inert to enzymatic hydrolysis) served to establish the β-glucoside linkage in these compounds. The derived aglycones, A2—4, were obtained as viscous oils and have the following spectral characteristics:† A2, $C_{15}H_{22}O_2$,‡ ν_{max} 3480, 3080, 1680, 1645, 1612, and 890 cm^{-1}; λ_{max} 240 nm (ε 13,800); δ 5·83 (1H, q, *J* 1·5 Hz), 4·74br (2H, s), 3·82 (1H, d, *J* 12·5 Hz), 2·03 (3H, d, *J* 1·5 Hz), 1·76br (3H, s), and 1·22 (3H, d, *J* 7 Hz); A3, $C_{15}H_{22}O_2$,‡ ν_{max} 3620, 3450, 3080, 1676, 1615, and 890 cm^{-1}; λ_{max} 241 nm (ε 13,000); δ 5·76br (1H, s), 5·07br (1H, s), 4·95br (1H, s), 4·14br (2H, s), 1·95 (3H, d, *J* 1 Hz), and 0·99 (3H, d, *J* 7 Hz); A4, $C_{15}H_{24}O_3$,‡ ν_{max} 3620, 3450, 1670, and 1610 cm^{-1}; λ_{max} 240 nm (ε 11,800); δ 5·75br (1H, s), 3·50 (2H, ABq, *J* 11 Hz), 1·97 (3H, d, *J* 1 Hz), 1·25 (3H, s), and 0·97 (3H, d, *J* 7 Hz). The most striking feature of the natural abundance ^{13}C n.m.r. spectra of A2—4 (Table) is the close similarity with the corresponding data[3] for (−)-solavetivone (**1**).§ On the basis of an analysis of the single-frequency off-resonance decoupled spectra, together with the spectral data noted above, A2—4 were assigned structures (**2**), (**3**),



SCHEME. Reagents: i, Ac_2O, pyridine (py); ii, Ca, NH_3 (ref. 5); iii, $LiNPr^i_2$, tetrahydrofuran (THF), −30 °C (ref. 6); iv, Me_3SiCl, Et_3N; v, *m*-$ClC_6H_4CO_3H$, −10°C (ref. 7); vi, aq. HCl; vii, $LiNPr^i_2$, THF, −70 °C; viii, MoO_5·py·hexamethylphosphoramide (ref. 8); ix, OsO_4; x, Na_2SO_3; xi, Ag_2CO_3, 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide; xii, aq. $KHCO_3$.

† The i.r., u.v., and n.m.r. spectra were recorded in CCl_4, EtOH, and $CDCl_3$ (+ Me_4Si) solutions respectively; the ^{13}C n.m.r. spectra of the glucosides were recorded in $(CD_3)_2SO$ solutions.

‡ Precise mass measurement.

§ Absolute stereochemistry is shown for convenience (*vide infra*).

TABLE

Assignment of ^{13}C n.m.r. shifts of (1)–(3) and (6)

Compound	C-1	C-2	C-3	C-4	C-5	C-6	C-7	C-8	C-9	C-10	C-11	C-12	C-13	C-14	C-15
(1)[3]	40·9	46·6	32·7	34·3	50·2	166·1	125·4	198·4	43·1	39·3	146·8	108·9	20·8	21·2	15·9
(2)	40·7	47·4	31·5	32·8	51·7	172·3	122·0	199·4	74·0	48·0	147·0	109·1	21·5	22·6	12·2
(3)	41·3	42·6	33·2	34·3	50·1	166·3	125·1	199·0	43·0	39·3	150·9	108·6	65·8	20·9	15·9
(6)	36·2	46·0	28·2	33·7	50·1	166·2	125·5	199·2	42·3	38·7	73·5	69·4	21·9	20·9	15·8

and (6)§ respectively. The relative stereochemistry at C-9 and C-10 in (2) was unambiguously determined from the n.m.r. spectrum in the presence of $Eu(fod)_3$ which clearly showed an AMX_3 pattern for the protons attached to C-9, C-10, and C-15 with $J_{9,10}$ 12·5 and $J_{10,15}$ 7 Hz thus suggesting that the hydroxy and methyl groups are pseudo-diequatorial in the cyclohexenone ring (7). In contrast, the lanthanide-shifted n.m.r. spectrum of (3) showed the C-9 protons as part of an ABX system (J_{AB} 17, J_{AX} and J_{BX} 4·5 Hz) thus implying that, without the hydrogen bonding influence in (7) the cyclohexenone ring in (3) adopts the other half-chair conformation (8) thereby minimising the *gauche* interaction between the C-10 methyl group and the cyclopentane ring.[4]

To consolidate the inter-relationship between (−)-solavetivone (1) and the aglycones (2) and (6), the following transformations were carried out (Scheme). (−)-Solavetivone (1), obtained from (2), was identical to the naturally occurring compound by g.l.c., h.p.l.c., t.l.c., m.s., ^1H and ^{13}C n.m.r., and optical rotation comparisons. The minor epimer (4) from both hydroxylation reactions of (−)-solavetivone (1)¶ is almost certainly the aglycone A1 which was unobtainable from G1 [the n.m.r. spectrum of (4) displayed a similar spectrum to (2) except that the C-9 proton resonated at lower field (δ 4·46, d, J 5 Hz) and the C-10 methyl group at higher field (δ 0·83, d, J 7 Hz)]. This point was reinforced by the preparation and separation of synthetic G1 and G2 from the above mixture of aglycones. In addition the ^{13}C n.m.r. spectra of G1 and G2 were similar. The glucosides were identical to the naturally occurring ones.

Although these experiments serve to define the stereochemical identity of the tobacco constituents at C-2, C-5, and C-10 with respect to (−)-solavetivone, the absolute configuration of the latter compound has not been unambiguously established.** In view of the considerable interest in sesquiterpenoid stress compounds from the *Solanaceae* species[9] and the fact that the absolute stereochemistry of only two of these (9) and (10) has been established, an X-ray analysis of (5) (m.p. 79—80 °C), the *p*-bromobenzenesulphonate of (2), was carried out.[10] *Crystal data:* $C_{21}H_{24}BrO_4S$, monoclinic, $P2_1$, $Z = 2$, $a = 14{\cdot}12$, $b = 6{\cdot}03$, $c = 13{\cdot}07$ Å, $\beta = 111{\cdot}10°$; 2257 observed reflexions from two axes on a STADI-2 diffractometer (Mo-K_α radiation) were collected. Heavy atom solution; all non-hydrogen atoms anisotropic. Present R 0·057 [for absolute configuration (2)]; R 0·085 for enantiomeric configuration.†† This result not only establishes the absolute configuration of solavetivone and the four new sesquiterpenoids described herein, but it also places the overall biogenetic relationships[9] of the ever-increasing number of *Solanaceae* sesquiterpenoids on a firmer footing. The precise genesis of the tobacco sesquiterpenoids is uncertain at the present time since any number of factors, *e.g.*, harvesting, curing, infection, *etc.*, could contribute to their production.

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¶ (−)-Solavetivone was isolated from *Phytophthora infestans* infected potato tubers; we thank Drs. D. T. Coxon and J. Malcolmson for their advice concerning the inoculation and isolation procedures. Lithium in ammonia reduction of (+)-anhydro-β-rotunol, prepared from (+)-nootkatone by the method of D. Caine and C.-Y. Chu, *Tetrahedron Letters*, 1974, 703, produced all four possible diastereoisomers in the ratio of 3 : 3 : 1 : 1 of which one of the minor components proved to be identical to (−)-solavetivone.

** Although the c.d. spectrum of (−)-solavetivone exhibits a positive Cotton effect, $\Delta\epsilon$ +0·53 at 316 nm, it was felt that assignment of absolute configuration based on this result would be suspect owing to lack of analogies. We thank Dr. P. M. Scopes for this measurement.

†† The atomic co-ordinates for this work are available on request from the Director of the Cambridge Crystallographic Data Centre, University Chemical Laboratory, Lensfield Road, Cambridge, CB2 1EW. Any request should be accompanied by the full literature citation for this communication.

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