Fluorinated tropane alkaloids generated by directed biosynthesis in transformed root cultures of *Datura stramonium*

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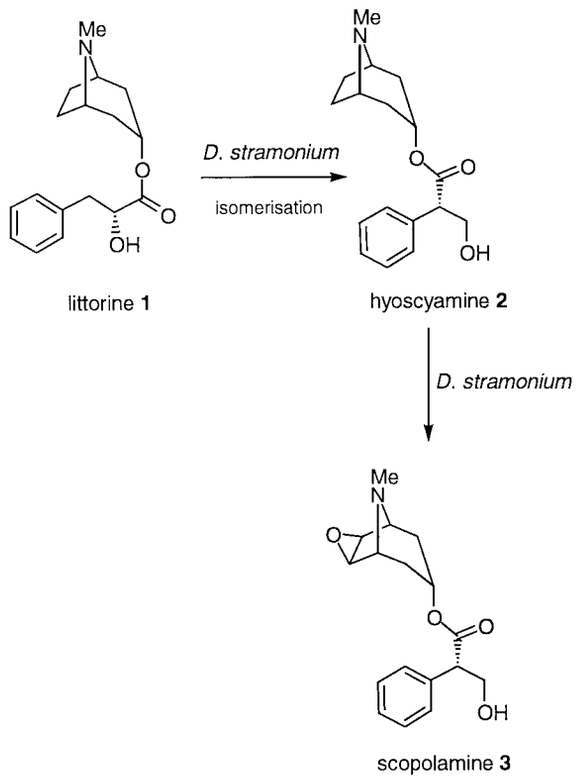
2′-, 3′- and 4′-Fluorophenyl-(*RS*)-lactic acids were administered to transformed root cultures of *Datura stramonium* to determine their abilities as substrates for incorporation into the phenyllactoyl and tropoyl ester moieties of the tropane alkaloids, littorine and hyoscyamine respectively. In the event, all of the fluorinated phenyllactates generated the corresponding fluorinated littorine and hyoscyamine analogues. The efficiency of conversion for the isomerisation of the fluorinated littorines (F-lit) to fluorinated hyoscyamines was 3′-F-lit > 4′-F-lit ⩾ 2′-F-lit.

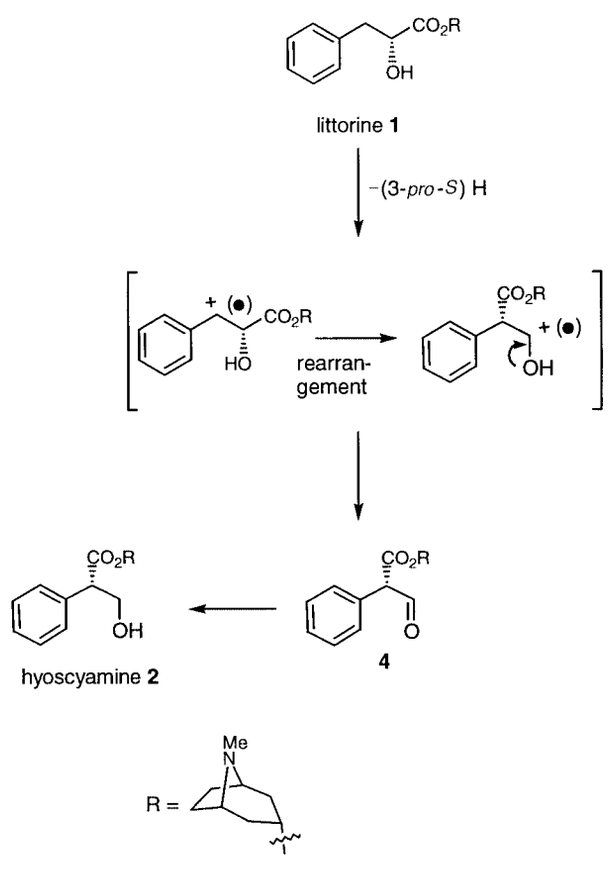
Introduction

The origin of the tropic acid ester moiety of the tropane alkaloids hyoscyamine **2** and scopolamine **3** (Scheme 1) has been the subject of biosynthetic interest for many years,[1] particularly as the tropate moiety was shown over twenty years ago[2] to arise from an intramolecular rearrangement of a phenylalanine derived metabolite. Recently it was discovered[3] that littorine **1**, the tropanyl ester (*R*)-phenyllactic acid is the substrate for this rearrangement and that littorine is converted to hyoscyamine in an intriguing biosynthetic isomerisation.[3] The mechanism of the rearrangement is unclear at present but our working hypothesis envisages the removal of the 3-*pro*-(*R*) hydrogen[4] by the putative isomerase to generate either a carbocation or radical at the benzylic C-3 position, and this intermediate undergoes rearrangement to a product carbocation or radical as shown in Scheme 2.

In a recent[5] oxygen-18 isotopic labelling study it was demon-



Scheme 1

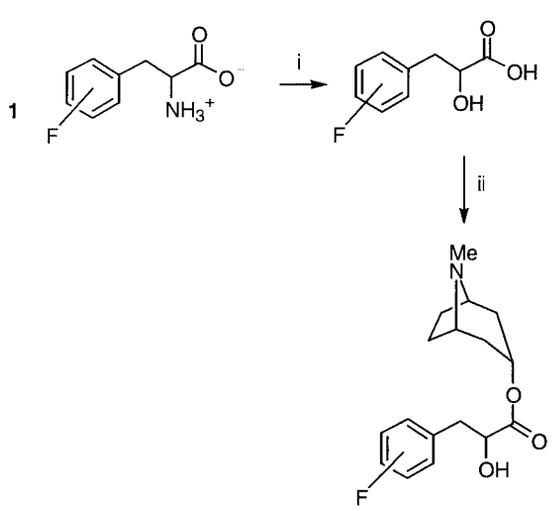


Scheme 2

strated that ~25–29% of the oxygen from the hydroxy oxygen at C-2 of littorine is lost in the rearrangement to hyoscyamine. One rationale for this leakage of oxygen-18 is the intermediacy of aldehyde **4** formed after collapse of the product carbocation, which would be vulnerable to loss of the isotope by exchange with the aqueous medium. The aldehyde would then require to be reduced by a dehydrogenase to generate littorine. In order to explore the littorine to hyoscyamine rearrangement further, 2′-, 3′-, and 4′-fluorophenyllactates were administered to root cultures of *Datura stramonium*. It was envisaged that these substrates, where each of the aromatic hydrogen atoms is in turn replaced by a fluorine atom, would be sufficiently similar to phenyllactate to be assimilated by the cells and become esterified to the corresponding fluorinated littorines. However the particular focus of this study was to test whether these fluorinated littorines would act as substrates for isomerisation to the equivalent fluorinated hyoscyamines. From classical substituent effects it was anticipated that the position of the fluorine would influence the stability of the putative intermediate benzylic carbocation or radical involved in the isomerisation process.

Results and discussion

Racemic samples of 2′-, 3′- and 4′-fluorophenyllactic acids were prepared by diazotization of the corresponding fluorinated phenylalanines in aqueous acidic conditions (Scheme 3).



Scheme 3 i, $NaNO_2$, cHCl, H_2O; ii, dry HCl(g), tropine.

It was also important for the study to prepare each of the corresponding fluorinated littorines to use as reference compounds for GC-MS analysis. These fluorinated littorines were prepared in an identical manner to that described[3] previously for the synthesis of littorine from phenyllactic acid and tropine. The fluorinated phenyllactic acids were administered at a final concentration of 0.1 mmol to transformed root cultures of *Datura stramonium*,[6] growing in a nutrient medium. This concentration was selected following a recent study in our laboratory where it was demonstrated that supplementation with phenyllactate[7] at this concentration stimulated littorine levels by approximately 35%. Higher concentrations produced no additional increase in littorine levels and this level of supplementation proved to be optimal. Cultures were fed in separate experiments with the fluorophenyllactate isomers on day 7. They were then worked up on days 11, 14 and 17 and the tropane alkaloids isolated as previously described[6] and the alkaloid mixtures analysed by GC-MS.[8,9] Analysis of the chromatographs revealed, as expected, littorine and hyoscyamine production as well as two additional but resolvable peaks indicating compounds closely related to these alkaloids. In the three cases studied, these two new peaks have molecular ions of $M^+ = 307$, characteristic of monofluorinated analogues of littorine and hyoscyamine. The fluorinated littorines were unambiguously identified by co-elution on GC and identical fragmentation patterns in GC-MS, with the appropriate synthetic reference compound. In all of the analyses, the fluorinated littorines proved to be the earlier eluted of the two new peaks. The latter peak was then assigned to the fluorinated hyoscyamine analogue.

Table 1 Relative ratios of littorine (lit), hyoscyamine (hyo), 2′-, 3′- and 4′-fluorolittorines (F-lit) and 2′-, 3′- and 4′-fluorohyoscyamines (F-hyo) after feeding experiments with 2′-, 3′- and 4′-fluorophenyllactates to *D. stramonium* root cultures. Values in the Table are ratios of two metabolites derived from GC integration values after feeding experiments worked up on days 11, 14 and 17

Precursor fed		Day 11	Day 14	Day 17
Control	hyo/lit	2.72	4.07	3.93
2′-F-pla	hyo/lit	2.97	3.08	3.07
	F-hyo/F-lit	0.09	0.14	0.08
	F-lit/lit	0.26	0.28	0.20
	F-hyo/hyo	0.01	0.01	0.01
3′-F-pla	hyo/lit	3.07	3.12	3.02
	F-hyo/F-lit	1.15	1.26	1.60
	F-lit/lit	0.13	0.11	0.09
	F-hyo/hyo	0.05	0.05	0.05
4′-F-pla	hyo/lit	2.60	2.73	3.19
	F-hyo/F-lit	0.40	0.42	0.62
	F-lit/lit	0.24	0.23	0.22
	F-hyo/hyo	0.04	0.04	0.04

Table 2 *In vivo* conversions (% values) of fluorolittorines to fluorohyoscyamines after biotransformations of F-phenyllactates in *D. stramonium* root cultures in experiments worked up after days 11, 14 and 17. The conversions are percentage values relative to the endogenous conversions of littorine to hyoscyamine in the same experiments. The data emerge from Table 1 as (F-hyo/F-lit)/(hyo/lit) × 100

	Day 11	Day 14	Day 17
2′-F-lit to 2′-F-hyo	3	4.5	2.6
3′-F-lit to 3′-F-hyo	37	40	53
4′-F-lit to 4′-F-hyo	15	15.3	19.4

The relative abundances of these compounds from each of the three experiments is presented in Table 1. For the three fluorophenyllactates studied, both fluorolittorines and fluorohyoscyamines were generated *in vivo*. The F-lit/lit ratios in Table 1 give an approximate measure of the 'incorporation' of exogenous fluorophenyllactic acid into the littorine pool (lit + F-lit). For 2′- and 4′-fluorophenyllactates the 'incorporation' levels reach about 25%. For 3′-fluorophenyllactate the incorporation level is lower at about 10%. On this basis, 3′-fluorophenyllactate appears to be a less efficient substrate for the esterification with tropine to generate the fluorinated littorine, however the levels of 3′-fluorolittorine in the extracts will be attenuated by the much higher *in vivo* conversions of 3′-F-lit to 3′-F-hyo relative to the other two isomers of F-lit (see below).

For the conversion of fluorolittorines to fluorohyoscyamines the incorporations are all low, and in the range 1 to 5% (see F-hyo/hyo levels in Table 1). To assess which of the fluorinated littorines was most efficiently converted to a fluorinated hyoscyamine, the F-hyo/F-lit ratio was evaluated as a percentage of the endogenous hyo/lit ratio in each experiment. This was judged to offer the most accurate measure of the efficiency of conversion of F-lit to F-hyo from the available data. These relative % conversions are presented in Table 2. It emerges that the efficiency of the biotransformation of the fluorolittorines to fluorohyoscyamines is in the order 3′-F-lit > 4′-F-lit ⩾ 2′-F-lit. For 3′-F-lit and 4′-F-lit there is an increase in the level of conversion from day 11 to day 17 whereas for 2′-F-lit the conversions are sufficiently low that this trend is not apparent. The

very low conversion of 2′-F-lit to 2′-F-hyo can most probably be attributed to the proximity of the fluorine atom to the benzylic reaction centre for the isomerisation and clearly the inductive influence of fluorine will be greatest at the 2-position. The results however do not reveal a progressive inductive effect for substitution at the other sites as 3′-F-lit emerged as a better substrate than 4′-F-lit for the isomerisation. From classical studies on fluorine substituent[10] effects, the intermediacy of a benzylic carbocation or radical is predicted to derive greater stabilisation from fluorine at the 4′-position over the 3′-position due to both inductive and mesomeric conjugative effects. However the relative incorporations of 3′- and 4′-fluorophenyllactates into hyoscyamine show the presence of a contrary effect which is not so straightforward to interpret. Perhaps significant differences in binding affinities to the isomerase between isomers are also contributing to this overall profile. To unravel this problem, it will be necessary to assess the kinetics of these fluorinated substrates with a purified isomerase, if and when such a system is forthcoming.

In summary it is demonstrated that 2′-, 3′- and 4′-fluorophenyllactates are esterified with tropine in *D. stramonium* root cultures and that the resultant fluorinated littorines are isomerised to the corresponding fluorinated hyoscyamines. The relative efficiencies of these conversions relate both to electronic effects and most probably binding affinities to the relevant enzymes.

Experimental

General details

IR spectra were recorded on a Perkin-Elmer F.T. 1720X or 1600 spectrometer. Mass spectra were recorded on a VG Analytical 7070E Organic mass spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Gemini 200 MHz (^{1}H at 199.975 MHz, ^{13}C at 50.289 MHz) and Varian VXR 400(S) (^{1}H at 399.952 MHz, ^{13}C at 100.577 MHz) instruments and ^{19}F NMR spectra were recorded on a Varian VXR 400(S) (^{19}F at 376.35 Hz) instrument. Chemical shifts are quoted relative to TMS (Me_4Si) in $CDCl_3$ or CD_3OD. GC-MS were recorded on a VG TRIOS-1S mass spectrometer (VG Masslab Ltd., Manchester) fitted with a Hewlett Packard 5890 series II gas chromatograph (Hewlett Packard Inc., Fort Collins, USA) and a DB-17 column (J&W Scientific, Folsom, USA) was used for separation. The three monofluorinated (*RS*)-phenylalanines were purchased from the Aldrich Chemical Company (Gillingham, Kent, UK) or Lancaster Synthesis Ltd., Morecambe, Lancashire, UK.

Root cultures of *Datura stramonium* L. D15/5 were grown as previously described.[6]

Alkaloids were extracted and analysed by GC-MS essentially as described previously.[8,9]

(*RS*)-3-(4′-Fluorophenyl)lactic acid

Conc. HCl (6.9 ml, 3 equiv.) was added to a solution of (*RS*)-3-(4-fluorophenyl)alanine (4.98 g, 27.2 mmol) in water (260 ml), and the reaction mixture stirred until homogenous. The solution was then cooled to 0 °C and $NaNO_2$ (3.90 g, 56.5 mmol, 2 equiv.) was added portion-wise over 4 h maintaining 0 °C. The reaction was left to come to ambient temperature and stirred for a further 48 h. The solution was concentrated under reduced pressure and extracted into ether (3 × 150 ml). The combined organic extracts were dried over $MgSO_4$, filtered and evaporated under reduced pressure to yield a yellow oil. Recrystallisation of the initial product from chloroform gave (*RS*)-3-(4′-fluorophenyl)lactic acid as a white crystalline solid (1.25 g, 25%). Mp 100.5–101.5 °C; $\delta_H(CD_3OD)$ 7.26 (dd, $^4J_{HF}$ = 5.6 Hz, $^3J_{HH}$ = 8.0 Hz, 2H, H-2′, H-6′), 6.98 (t, $^3J_{HF,\ HH}$ = 8.8 Hz, 2H, H-3′, H-5′), 4.30 (dd, ^3J = 4.4 Hz, ^3J = 8.0 Hz, 1H, -CH), 3.07 (dd, ^3J = 4.4 Hz, ^2J = 14.0 Hz, 1H, -C*H*H), 2.88 (dd, ^3J = 8.0 Hz, ^2J = 14.0 Hz, 1H, -CH*H*); $\delta_C(CD_3OD)$ 177.0 (C-1), 72.6 (C-2), 40.6 (C-3), 163.2 (d, $^1J_{CF}$ = 242.6 Hz, C-4′), 134.8 (d, $^4J_{CF}$ = 2.9 Hz, C-1′), 132.3 (d, $^3J_{CF}$ = 8.0 Hz, C-2′, C-6′), 115.7 (d, $^2J_{CF}$ = 21.3 Hz, C-3′, C-5′); $\delta_F(CD_3OD)$ −119.6 (m, 1F, Ar-F); *m*/*z* (EI+) 184 (M^+, 6.55%), 166 ($M^+ - 18$, 20.97), 109 ($M^+ - 75$, 100); ν_{max} 3417, 2950, 1702, 1600, 1507, 1445, 1417, 1363 and 1228 cm^{-1} (Calcd. mass for $C_9F_1H_9O_3$: 184.053573. Found: 184.053398).

(*RS*)-3-(3′-Fluorophenyl)lactic acid

The above procedure for (*RS*)-3-(4′-fluorophenyl)lactic acid was repeated using (*RS*)-3-(3′-fluorophenyl)alanine (4.83 g, 26.4 mmol) to yield a yellow oil which crystallised after addition of chloroform to give the product as a white crystalline solid (1.21 g, 25%). Mp 106–107 °C; $\delta_H(CD_3OD)$ 7.27 (dt, $^4J_{HF}$ = 6.8 Hz, $^3J_{HH}$ = 7.6 Hz, 1H, H-5′), 7.07 (d, $^3J_{HH}$ = 7.6 Hz, 1H, H-6′), 7.02 (d, $^3J_{HH}$ = 10.0 Hz, 1H, H-2′), 6.93 (t, $^3J_{HF,\ HH}$ = 8.8 Hz, 1H, H-4′), 4.34 (dd, ^3J = 4.4 Hz, ^3J = 8.0 Hz, 1H, -CH), 3.10 (dd, ^3J = 4.0 Hz, ^2J = 14.0 Hz, 1H, -C*H*H), 2.91 (dd, ^3J = 8.0 Hz, ^2J = 14.0 Hz, 1H, -CH*H*); $\delta_C(CD_3OD)$ 176.8 (C-1), 164.1 (d, $^1J_{CF}$ = 243.8 Hz, C-3′), 141.7 (d, $^3J_{CF}$ = 7.3 Hz, C-1′), 130.8 (d, $^3J_{CF}$ = 8.0 Hz, C-5′), 126.5 (d, $^4J_{CF}$ = 2.6 Hz, C-6′), 117.3 (d, $^2J_{CF}$ = 21.3 Hz, C-2′), 114.2 (d, $^2J_{CF}$ = 22.4 Hz, C-4′), 72.4 (C-2), 41.2 (C-3); $\delta_F(CD_3OD)$ −116.6 (m, 1F, Ar-F); *m*/*z* (EI+) 184 (M^+, 3.37%), 166 ($M^+ - 18$, 32.26), 109 ($M^+ - 75$, 100); ν_{max} 3428, 2940, 1722, 1585, 1486, 1450, 1429, 1324, 1240 cm^{-1} (Calcd. mass for $C_9F_1H_9O_3$: 184.053573. Found: 184.053243).

(*RS*)-3-(2′-Fluorophenyl)lactic acid

The above procedure for (*RS*)-3-(4′-fluorophenyl)lactic acid was repeated using (*RS*)-(3)-2′-fluorophenylalanine (5.09 g, 27.8 mmol) to yield a yellow oil which crystallised after addition of chloroform to give the product as a white crystalline solid (0.957 g, 19%). Mp 80–81 °C; $\delta_H(CD_3OD)$ 7.29–7.32 (m, 1H, Ar-H), 7.20–7.24 (m, 1H, Ar-H), 7.02–7.09 (m, 2H, Ar-H), 4.34 (dd, ^3J = 4.4 Hz, ^3J = 7.6 Hz, 1H, -CH), 3.17 (dd, ^3J = 3.2 Hz, ^2J = 14.0 Hz, 1H, -C*H*H), 2.91 (dd, ^3J = 8.4 Hz, ^2J = 13.2 Hz, 1H, -CH*H*); $\delta_C(CD_3OD)$ 176.9 (C-1), 71.6 (C-2), 34.9 (C-3), 162.8 (d, $^1J_{CF}$ = 244.1 Hz, C-2′), 133.1 (d, $^3J_{CF}$ = 4.2 Hz, C-6′), 129.5 (d, $^3J_{CF}$ = 8.0 Hz, C-4′), 125.8 (d, $^2J_{CF}$ = 15.7 Hz, C-1′), 125.0 (d, $^4J_{CF}$ = 3.0 Hz, C-5′), 116.0 (d, $^2J_{CF}$ = 22.1 Hz, C-3′); $\delta_F(CD_3OD)$ −120.4 (m, 1F, Ar-F); *m*/*z* (EI+) 184 (M^+, 2.47%), 166 ($M^+ - 18$, 23.19), 109 ($M^+ - 75$, 100); ν_{max} 3416, 2950, 1703, 1604, 1507, 1450, 1418, 1362, 1230 cm^{-1} (Calcd. mass for $C_9F_1H_9O_3$: 184.053573. Found: 184.053840).

Tropanyl (*RS*)-3-(4′-fluorophenyl)lactate (4′-F-littorine)

Rigorously dried (*RS*)-3-(4′-fluorophenyl)lactic acid (390 mg, 2.1 mmol) and tropine (270 mg, 1.9 mmol) were mixed intimately under N_2 in the solid phase. The mixture was heated to 130 °C and a current of dry HCl gas was passed periodically over the reaction for 5 h and the reaction mixture was allowed to cool to ambient temperature. The product was dissolved as completely as possible in 0.05 M H_2SO_4 (10 ml), filtered and the filtrate was treated with 10% aqueous ammonium hydroxide until it was basic, and then the organics were extracted into chloroform (4 × 15 ml), the extracts dried ($MgSO_4$) and concentrated under reduced pressure to give a pale yellow solid, (367 mg, 57%). Mp 77–79 °C; $\delta_H(CDCl_3)$ 7.20 (dd, $^4J_{HF}$ = 5.6 Hz, $^3J_{HH}$ = 8.8 Hz, 2H, H-2′, H-6′), 6.99 (t, $^3J_{HF,\ HH}$ = 8.8 Hz, 2H, H-3′, H-5′), 5.06 (t, ^3J = 5.2 Hz, 1H, H-3), 4.36 (dd, ^3J = 4.8 Hz, ^3J = 7.2 Hz, 1H, -CH), 3.10 (dd, ^3J = 4.8 Hz, ^2J = 14.0 Hz, 1H, -C*H*H), 3.10–3.18 (br, 2H, H-1, H-5), 2.95 (dd, ^3J = 6.8 Hz, ^2J = 14.0 Hz, 1H, -CH*H*), 2.30 (s, 3H, NMe), 2.12–2.26 (m, 2H, H-2$_e$, H-4$_e$), 1.96–2.04 (m, 2H, H-6$_e$, H-7$_e$), 1.74–1.85 (m, 2H, H-7$_a$, H-6$_a$), 1.67 (t, ^2J = 14.0 Hz, 2H, H-4$_a$, H-2$_a$); $\delta_C(CDCl_3)$ 173.2 (C=O), 162.0 (d, $^1J_{CF}$ = 245.3 Hz, C-4′), 132.0 (d, $^4J_{CF}$ = 3.4 Hz, C-1′), 130.9 (d, $^3J_{CF}$ = 7.9 Hz, C-2′, C-6′), 115.3 (d, $^2J_{CF}$ = 21.3 Hz, C-3′, C-5′), 71.3 (C-H′), 69.3 (C-3), 59.7

(C-1, C-5), 40.3 (NMe), 39.6 (C-HH′), 36.4 (C-2, C-4), 25.4 (C-6, C-7); $\delta_F(CDCl_3)$ −116.4 (m, 1F, Ar-F); *m/z* (EI+) 307 (M^+, 25.02%), 140 ($M^+ - 167$, 15.06), 124 ($M^+ - 183$, 100); ν_{max} 2944, 1730, 1601, 1508, 1448, 1418, 1218 cm^{-1} (Calcd. mass for $C_{17}F_1H_{22}NO_3$: 307.158372. Found: 307.158504).

Tropanyl (*RS*)-3-(3′-fluorophenyl)lactate (3′-F-littorine)

The above procedure for 4′-F-littorine was repeated using previously prepared (*RS*)-3-(3′-fluorophenyl)lactic acid (201 mg, 1.1 mmol) and tropine (155 mg, 1.1 mmol). The product was purified on a Hyflow column (10 g) containing phosphate buffer (0.5 mol dm^{-3}) at pH 6.6, eluting with successive volumes (50 ml) of light petroleum, ether and chloroform, to give 3′-F-littorine (51 mg, 15%) as a pale yellow gum. $\delta_H(CDCl_3)$ 7.22–7.29 (m, 1H, Ar-H), 6.90–7.02 (m, 3H, Ar-H), 5.05 (t, $^3J = 4.8$ Hz, 1H, H-3), 4.37 (dd, $^3J = 4.4$ Hz, $^3J = 6.8$ Hz, 1H, -CH), 3.11 (dd, $^3J = 4.4$ Hz, $^2J = 14.4$ Hz, 1H, -C*H*H), 3.11–3.16 (br, 2H, H-1, H-5), 2.96 (dd, $^3J = 7.2$ Hz, $^2J = 14.0$ Hz, 1H, -CH*H*), 2.29 (s, 3H, NMe), 2.14–2.24 (m, 2H, H-2_e, H-4_e), 1.98–2.04 (m, 2H, H-6_e, H-7_e), 1.79 (d, $J = 8.0$ Hz, 2H, H-7_a, H-6_a), 1.66 (t, $^2J = 16.4$ Hz, 2H, H-4_a, H-2_a); $\delta_C(CDCl_3)$ 173.4 (C=O), 163.0 (d, $^1J_{CF} = 245.7$ Hz, C-3′), 139.2 (d, $^3J_{CF} = 7.5$ Hz, C-1′), 130.1 (d, $^3J_{CF} = 8.3$ Hz, C-5′), 125.3 (d, $^4J_{CF} = 2.3$ Hz, C-6′), 116.6 (d, $^2J_{CF} = 21.0$ Hz, C-2′), 114.0 (d, $^2J_{CF} = 20.9$ Hz, C-4′), 71.4 (C-H), 69.5 (C-3), 60.0 (C-1, C-5), 40.5 (NMe), 40.4 (C-HH), 36.6 (C-2), 36.5 (C-4), 25.7 (C-6, C-7); $\delta_F(CDCl_3)$ −113.8 (m, 1F, Ar-F); *m/z* (EI+) 307 (M^+, 19.62%), 140 ($M^+ - 167$, 13.92), 124 ($M^+ - 183$, 100); ν_{max} 2948, 1740, 1620, 1592, 1492, 1452, 1254 cm^{-1} (Calcd. mass for $C_{17}F_1H_{22}NO_3$: 307.158372. Found: 307.158538).

Tropanyl (*RS*)-3-(2′-fluorophenyl)lactate (2′-F-littorine)

The above procedure for 4′-F-littorine was repeated using (*RS*)-3-(2′-fluorophenyl)lactic acid (180 mg, 0.98 mmol) and tropine (138 mg, 0.98 mmol). The product was purified on a Hyflow column (10 g) containing phosphate buffer (0.5 mol dm^{-3}) at pH 6.6, eluting with successive volumes (50 ml) of light petroleum, ether and chloroform to give 2′-F-littorine (42 mg, 14%) as a pale yellow oil. $\delta_H(CDCl_3)$ 7.20–7.30 (m, 2H, Ar-H), 7.00–7.11 (m, 2H, Ar-H), 5.01–5.10 (br, 1H, H-3_e), 4.40 (dd, $^3J = 5.2$ Hz, $^3J = 7.2$ Hz, 1H, -CH), 3.17 (dd, $^3J = 4.8$ Hz, $^2J = 14.0$ Hz, 1H, -C*H*H), 3.16–3.24 (br, 2H, H-1, H-5), 3.01 (dd, $^3J = 7.2$ Hz, $^2J = 13.6$ Hz, 1H, -CH*H*), 2.33 (s, 3H, NMe), 2.20–2.32 (m, 2H, H-2_e, H-4_e), 1.96–2.08 (m, 2H, H-6_e, H-7_e), 1.78–1.92 (m, 2H, H-7_a, H-6_a), 1.75 (d, $^2J = 14.4$ Hz, 1H, H-2_a), 1.65 (d, $^2J = 14.4$ Hz, 1H, H-4_a); $\delta_C(CDCl_3)$ 173.4 (C=O), 161.2 (d, $^1J_{CF} = 245.3$ Hz, C-2′), 131.9 (d, $^3J_{CF} = 4.2$ Hz, C-6′), 128.8 (d, $^3J_{CF} = 8.4$ Hz, C-4′), 124.1 (d, $^4J_{CF} = 3.1$ Hz, C-5′), 123.4 (d, $^2J_{CF} = 15.6$ Hz, C-1′), 115.3 (d, $^2J_{CF} = 22.1$ Hz, C-3′), 70.3 (C-H), 68.9 (C-3), 59.9 (C-1, C-5), 40.0 (NMe), 36.1 (C-2), 35.8 (C-4), 34.1 (C-HH), 25.3 (C-7), 25.1 (C-6); $\delta_F(CDCl_3)$ −118.2 (m, 1F, Ar-F); *m/z* (EI+) 307 (M^+, 23.67%), 140 ($M^+ - 167$, 12.47), 124 ($M^+ - 183$, 100); ν_{max} 2923, 1739, 1587, 1493, 1461, 1377, 1260 cm^{-1} (Calcd. mass for $C_{17}F_1H_{22}NO_3$: 307.158372. Found: 307.159058).

Feeding (*RS*)-3-(2′-fluorophenyl)lactic acid to *Datura stramonium* root cultures

To six subcultured flasks each containing an initial innoculum (0.5 g) of fresh mass of roots in culture medium (50 ml), a sterile solution (1 ml) of (*RS*)-3-(2′-fluorophenyl)lactic acid (54.3 mmol dm^{-3}) dissolved in MeOH was fed on day 7 at a final concentration of 0.1 mmol dm^{-3} in the medium. Two flasks each of roots were harvested and freeze-dried after days 11, 14 and 17. The freeze-dried roots (day 11, 0.6 g; day 14, 0.6 g; day 17, 0.6 g) were ground with acid-washed sand and extracted into H_2SO_4 (10 ml, 0.05 mol dm^{-3}) by stirring for 20 min. The aqueous extract was made basic with 35% NH_3 solution and then filtered through Hydromatrix® and eluted with $CHCl_3$–MeOH (20 : 1). The eluent was evaporated under reduced pressure to give a brown oil (day 11, 7 mg; day 14, 8 mg; day 17, 10 mg) containing the alkaloids and this extract was subjected to GC-MS analysis.

Feeding (*RS*)-3-(3′-fluorophenyl)lactic acid to *Datura stramonium* root cultures

The above procedure for (*RS*)-3-(2′-fluorophenyl)lactic acid was repeated for (*RS*)-3-(3′-fluorophenyl)lactic acid. The freeze-dried roots obtained (day 11, 0.5 g; day 14, 0.6 g; day 17, 0.5 g) yielded the alkaloid extract as a brown oil (day 11, 14 mg; day 14, 8 mg; day 17, 7 mg) which was subjected to GC-MS analysis.

Feeding (*RS*)-3-(4′-fluorophenyl)lactic acid to *Datura stramonium* root cultures

The above procedure for (*RS*)-3-(2′-fluorophenyl)lactic acid was repeated for (*RS*)-3-(4′-fluorophenyl)lactic acid. The freeze-dried roots obtained (day 11, 0.5 g; day 14, 0.6 g; day 17, 0.6 g) yielded the alkaloid extract as a brown oil (day 11, 10 mg; day 14, 9 mg; day 17, 10 mg) which was subjected to GC-MS analysis.

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