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TROPANE ALKALOIDS FROM *ANTHOCERCIS* AND *ANTHOTROCHE*

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Abstract—In addition to known tropane alkaloids, 3α-*iso*-butyryloxytropan-6β-ol and 3α-*n*-butyryloxytropane have been characterized as respective components of the aerial parts of two subspecies of *Anthocercis albicans*. Aponoratropine, not previously recorded as a natural product, has been detected in the leaves of *Anthotroche myoporoides* and *A. walcottii*; similarly aponorhyoscine is indicated as a component of the roots of a subspecies of *Anthocercis genistoides*.

INTRODUCTION

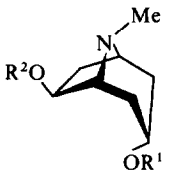
Anthocercis and *Anthotroche* are two closely related woody genera of the Australian Solanaceae and their taxonomy is currently under revision (L. Haegi, private communication). As part of a comprehensive chemotaxonomic survey involving the distribution of tropane alkaloids in the two genera, we report here the isolation of new alkaloids from four previously uninvestigated species. Tropane alkaloids are known to occur in *Anthocercis littorea*, *A. fasciculata*, *A. viscosa*[1, 2], *A. tasmanica*[3], *A. frondosa*[4] and in *Anthotroche pannosa*[5]. Nicotine has been reported as a component of *Anthocercis tasmanica*[3] and *A. frondosa*[4].

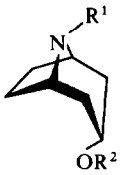
RESULTS AND DISCUSSION

The total alkaloids of *Anthocercis albicans*, representing 0.05% of the dried aerial parts, were separated into two fractions by column partition chromatography at pH 6.8. The principal components of the first ether fraction were identified as hyoscine, possibly valtropine, 3α-*iso*-butyryloxytropane and valeroidine; the second chloroform fraction contained small quantities of hyoscyamine, 3α-acetoxytropane and 3α-tigloyloxytropane, together with a new base which was purified by preparative TLC and constituted the major component of this second fraction.

The IR spectrum of the new base showed a OH (ν_{max} 3515 cm^{-1}) and an ester carbonyl (ν_{max} 1729 cm^{-1}). Mass spectrometry gave the molecular formula $C_{12}H_{21}NO_3$ together with the well-established characteristic fragmentation pattern [6] of a monoester of tropane-3α,6β-diol. The ions at *m/e* 156 ($M^+ - 71$) and 140 ($M^+ - 87$) gave the esterifying acid as $C_4H_8O_2$ (*n*- or *iso*-butyric acid). Lack of a fragment at *m/e* 113 and the presence of an ion at *m/e* 183 indicated esterification at C-3 [6]. The base is therefore dihydroxytropane esterified at C-3 with either *n*-butyric or *iso*-butyric acid. NMR spectroscopy confirmed the presence of a free OH, and a doublet at δ 1.15 integrating for six protons was attributable to the protons of two terminal Me groups of an isopropyl moiety; a similar signal at δ 0.97 is present in the NMR spectrum of valeroidine (**1c**). (An *n*-butyryl ester would have afforded a characteristic uneven triplet resulting from coupling involving the methylene group of the side chain.) The identity of the acid was confirmed by alkaline hydrolysis of the base and GC of the liberated acid. The 3α-orientation of the esterifying group and the free β-orientated OH at C-6 were demonstrated by a triplet in the NMR spectrum at δ 4.95 and a quartet at δ 4.5, respectively [6]. Other features of the NMR spectrum were in accord with the suggested structure. The optical characteristics of the alkamine moiety were determined by hydrolysis of the base and esterification of the resulting diol (**1a**) with tigloyl chloride; the picrate of the diester (**1d**) produced, had mp and mmp with (+)-3α,6β-ditigloloxytropane picrate 149° and with the (−)-ester the mmp was raised to 171–173°. This established [7] the alkamine as (−)-3α,6β-dihydroxytropane, the same as for valeroidine (**1c**). The new base is therefore 3α-*iso*-butyryloxytropan-6β-ol (**1b**).

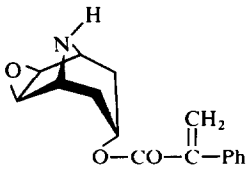
A subspecies (L. Haegi, private communication) of *Anthocercis albicans* (Haegi collection No. 1379) contained, in the dried leaves, 0.02% alkaloid calculated as hyoscyamine. Fractionation of the alkaloid mixture by column chromatography at pH 6.8 followed, where necessary, by preparative TLC gave apohyoscine, hyoscine (principal alkaloid), 3α-acetoxytropane and probably 3α-*iso*-butyryloxytropan-6β-ol and 3α-tigloyloxytropane. In addition, the major base of the chloroform eluate afforded a picrate, mp 191°, and was characterized as 3α-*n*-butyryloxytropane (**2a**), a base not previously isolated as a single compound from plant material. The IR spectrum of the picrate showed a strong ester carbonyl absorption at 1726 cm^{-1} and high resolution MS gave a M^+ corresponding to $C_{12}H_{21}NO_2$ with a fragmentation pattern corresponding to a simple tropanol ester [*m/e* 94, 124 (100%), 140]. The acyl moiety ($M^+ - 140$), mass 71, corresponds either to *n*-butyric acid or to *iso*-butyric acid. 3α-*n*-Butyryloxytropane (**2a**) and 3α-*iso*-butyryloxytropane [butropine (**2b**)] were prepared by partial synthesis and their picrates compared





1a $R^1 = R^2 = H$
1b $R^1 = Me_2CH{\cdot}CO$, $R^2 = H$
1c $R^1 = Me_2CH{\cdot}CH_2$, $R^2 = H$
1d $R^1 = R^2 =$ MeCH:CMeCO

2a $R^1 =$ Me, $R^2 = MeCH_2{\cdot}CH_2{\cdot}CO$
2b $R^1 =$ Me, $R^2 = Me_2CH{\cdot}CO$
2c $R^1 = H$, $R^2 = H_2C{:}C(Ph)CO$



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with that of the natural base. The picrate melting points of the *n*-butyryl ester and the natural derivative were 194° and 191°, respectively, with mmp 192–193°, both compounds possessed the same ester carbonyl absorption and the liberated bases had identical R_f values; on the other hand, butropine picrate melted at 214° and showed ν_{max} 1738 cm^{-1}. This is the first report of the isolation of 3α-*n*-butyryloxytropane from plant material although it has been previously synthesized [8] and its presence in *Bruguiera sexangula* (Rhizophoraceae) tentatively reported [9], *n*-butyric acid being among the components identified by GC after hydrolysis of a crude alkaloid extract of the plant.

The leaves of *Anthotroche myoporoides* and *A. walcottii* were similarly examined for alkaloids and both contained hyoscyamine, norhyoscyamine, apoatropine and hyoscine. In addition, in both species, a small proportion of a new alkaloid, MW 257, was evident and it was purified by preparative TLC. MS indicated a nortropane ester *m/e* 110 (100%), 80 (22%) with a C_8H_7 unit *m/e* 103 (16%) derived from the esterifying acid and given by all esters of atropic acid. Aponoratropine (**2c**), synthesized from noratropine sulphate by dehydration with sulphuric acid, had identical R_f values to those of the new base in three chromatographic systems and the picrates gave identical mass spectra. Although aponoratropine has not been previously reported in plant material, its occurrence in such species as the above is not unexpected because both norhyoscyamine and apoatropine are prominent constituents of the alkaloid mixture.

The alkaloid mixture of the roots of a subspecies (L. Haegi, private communication) of *Anthocercis genistoides* included hyoscine (principal alkaloid), apohyoscine, norhyoscine, meteloidine and possibly 6-hydroxyhyoscyamine. In addition, one fraction together with a similar one from the leaves contained a mixture of bases and possessed one alkaloid, MW 271 ($C_{16}H_{17}NO_3$); also TLC of the mixture showed one component with an R_f value identical to that of synthetic aponorhyoscine (MW 271) (**3**). Lack of material prevented purification of the natural base. Aponorhyoscine has not been reported before as a natural product but as with aponoratropine, its presence in small quantities in this group of plants is not unexpected.

The new bases reported here from *Anthocercis* and *Anthotroche* will provide data, additional to that from known bases, for the chemotaxonomic assessment of the two genera.

EXPERIMENTAL

Plant materials. Origins are given in Table 1.

Extraction and fractionation (column chromatography and TLC) of alkaloids. These followed the general methods previously described [2].

3α-Iso-*butyryloxytropan-6β-ol* (**1b**). The alkaloids from the aerial parts (250 g) of *Anthocercis albicans* were obtained in 2 fractions by partition chromatography (kieselguhr 10 g; 0.5 M phosphate buffer, pH 6.8, 3.5 ml). The first Et_2O fraction gave after prep. TLC (Si gel; $CHCl_3$–Et_2NH,9:1; Me_2CO–H_2O–NH_4OH,90:7:3) hyoscine (R_f, picrate, IR, MS), valeroidine in admixture (R_f picrate, IR, MS), 3α-*iso*-butyryloxytropane (R_f, picrate, IR, MS) and valtropine (MS). Prep. TLC (Si gel; Me_2CO–H_2O–NH_4OH, 90:7:3) of the second, $CHCl_3$, fraction gave small quantities of hyoscyamine, 3α-acetoxytropane, 3α-tigloyloxytropane (R_f values, 4 systems) together with a major component which with Na picrate soln afforded 3α-*iso*-butyryloxytropan-6β-ol picrate, plates from 50% EtOH, mp 162–163°. (Found: C, 47.2; H, 4.7. $C_{12}H_{21}NO_3{\cdot}C_6H_3N_3O_7$ requires: C, 47.4; H, 5.3%); MS *m/e* (rel. int.): 227 (M^+), 183, 156, 141, 140 (33), 122, 112, 110, 109, 108, 96 (13), 95 (67), 94 (100), 82 (11). Accurate mass measurement for M^+ = 227.1534, $C_{12}H_{21}NO_3$ requires 227.1522. NMR, base regenerated from picrate in $CDCl_3$ with TMS as int. standard, δ 1.15 (6H, *d*, ββ methyls), 2.5 (3H, *s*, NMe), 3.15 (1H, *s*, C-6 hydroxyl), 4.5 (1H, *q*, C-6), 4.95 (1H, *t*, C-3). Hydrolysis [7] of the base (12 mg) afforded *iso*-butyric acid as the principal acid component identified by GC (adjusted R_t and R_t of authentic compound 2.3 min, R_t of *n*-butyric acid 3.2 min on

Table 1. Plant materials

Species	Australian locality	Date collected	Collector and No.	Herbarium deposition
Anthocercis albicans A. Cunn.	*ca* 45 km NE of Hillston, N.S.W.	Sept. 1977	Haegi 1363	NSW
A. albicans A. Cunn. ssp. 1	*ca* 10 km W. of Coonabarabran, N.S.W.	Sept. 1977	Haegi 1379	NSW
A. genistoides Miers ssp. 1	*ca* 15 km SW of Ravensthorpe, W.A.	Sept. 1976	Haegi 1036	AD
Anthotroche myoporoides C.A. Gardn.	*ca* 70 km NE Geraldton, W.A.	Sept. 1976	Haegi 1156	AD
A. walcottii F. Muell.	*ca* 38 km NW of Ajana, W.A.	Sept. 1976	Haegi 1153	AD

PEGA 10%, 126°, 1.75 m glass column, flow rate of carrier 50 ml/min). The alkamine fraction of the hydrolysate, having R_f values identical to those of tropan-3α,6β-diol was characterized as indicated earlier. Esterification of the natural base (2 mg) with one equivalent of *iso*-butyryl chloride gave a product having IR and MS (M^+ 297.1950; $C_{16}H_{27}NO_4$ requires 297.1941) identical to those of synthetic 3α,6β-di-*iso*-butyryloxytropane prepared from the (−)-diol.

3α-n-*Butyryloxytropane* (**2a**). Leaves (264 g) of a subspecies of *Anthocercis albicans* were extracted and the alkaloids fractionated and characterized as above. In addition to known alkaloids, the first portion of the $CHCl_3$ eluate contained a major component which, when purified by prep. TLC (Si gel; $CHCl_3$–Et_2NH, 9:1), afforded after treatment with aq. Na picrate soln 3α-*n*-butyryloxytropane picrate needles from 50% aq. EtOH, mp 191°; accurate mass measurement of M^+ = 211.1562, $C_{12}H_{21}NO_2$ requires 211.1572; λ_{max} 1726 cm^{-1} (ester CO). MS *m/e* (rel. int.): 211 (M^+), 140 (14) [140.1080; calc. for $C_8H_{14}NO$: 140.1076], 125 (15), 124 (100), 96 (31), 95 (14), 94 (62), 83 (39), 82 (72). The synthetic ester gave a picrate, mp 194°. (Found: C, 48.8; H, 5.44; N, 12.5. Calc. for $C_{12}H_{21}NO_2 \cdot C_6H_3N_3O_7$: C, 49.1; H, 5.45; N, 12.7%). Mmp with the picrate of the natural base 192–193°; both had the same spectroscopic characteristics. The product [butropine **2b**] of the esterification of tropine and isobutyryl chloride gave a picrate, mp 214° (lit. 224° [8]), λ_{max} 1738 cm^{-1}; MS *m/e* (rel. int.): 211 (M^+) (211.1547; calc. for $C_{12}H_{21}NO_2$: 211.1572), 140 (29), 125 (16), 124 (100), 96 (37), 94 (51), 83 (63), 82 (98).

Aponoratropine (**2c**). Known bases from the aerial parts of *Anthotroche myoporoides* (200 g) and *A. walcottii* (190 g) were extracted and characterized as above. Fractions from the two plants which contained in admixture a new base (MW 257) were combined and rechromatographed (Al_2O_3; Et_2O–EtOH, 1:1). A base, R_f 0.07, recovered as the picrate gave MS (principal ions) *m/e* 257 (10) M^+, 111 (14), 110 (100), 109 (12), 103 (16), 91 (8), 83 (14), 82 (14), 81 (18), 80 (22), 77 (12), 73 (14), 72 (12), 71 (10), 69 (18), 68 (22), 67 (20), 58 (60), 57 (20), 56 (10), 55 (14). Aponoratropine picrate, synthesized from noratropine sulphate (50 mg) by the method of ref. [10] for apohyoscine, had the same MS characteristics and R_f values in 3 systems as the natural base; *m/e* 257.1435; $C_{16}H_{19}NO_2$ requires 257.1416; 110.0968, calc. for $C_7H_{12}N$: 110.0969; 103.0543, calc. for C_8H_7: 103.0547.

Aponorhyoscine (**3**). Prepared as above from norhyoscine, aponorhyoscine picrate gave plates, mp 224–225°, from aq. EtOH. MS *m/e* (rel. int.): 271 (19) M^+, 122 (100), 103 (85), 94 (59), 80 (94). The mixture of bases from the plant material gave *m/e* 271.1211, $C_{16}H_{17}NO_3$ requires 271.1208; other uncharacterized ions included 307.1778, calc. for $C_{17}H_{25}NO_4$: 307.1783, 325.1838, calc. for $C_{24}H_{23}N$: 325.1831 and other components were present.

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