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Absolute configuration assignment and enantiopurity determination of chiral alkaloids and coumarins derived from O- and C-prenyl epoxides†

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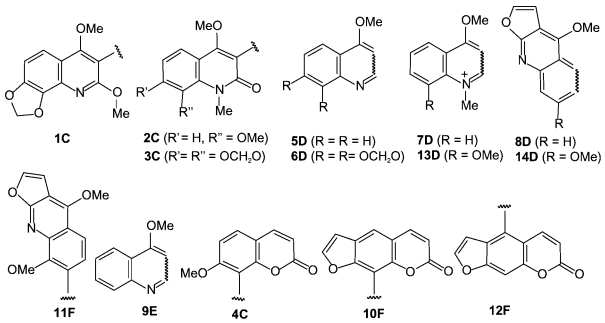
A combination method of ozonolysis and chiral stationary phase (CSP)-GC–MS analysis has been developed to determine the enantiopurity values and absolute configurations of a range of alkaloid and coumarin hemiterpenoids derived from C- and O-prenyl epoxides.

The biosynthesis of many hemiterpenoids including quinoline alkaloids and coumarins involves an enzyme-catalysed epoxidation of C-prenyl (**A**, R = Ar) and O-prenyl (**A**, R = O–Ar) groups to form the corresponding chiral prenyl (3,3-dimethylallyl) epoxides **B** (Scheme 1). The epoxide intermediates (**B**, R = Ar or OAr) may undergo further enzyme-catalysed (i) hydrolysis to yield 1,2-diols (**C** or **F**) or (ii) cyclisation (**B**, R = Ar) to yield dihydrofurans (**D**) and dihydropyrans (**E**).

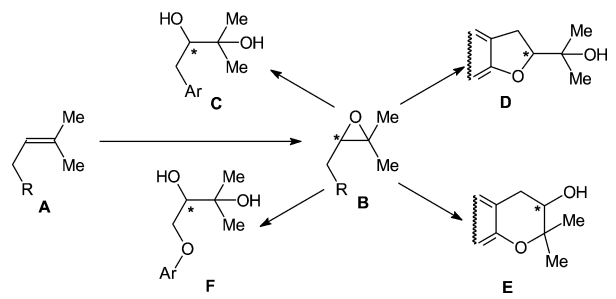
Individual quinoline alkaloids have exhibited anti-bacterial, anti-fungal, anti-viral, cytotoxic, phototoxic and mutagenic activities and an ability to form cycloadducts with DNA.[1] Isolation of new chiral quinoline alkaloids continues to be an area of interest.[1b] Our current synthetic studies, running concurrently with an investigation of the alkaloids of *Choisya ternata*, have yielded known and new quinoline alkaloids and coumarins. Synthetic routes to enantiopure quinoline alkaloids, based on the resolution of bromohydrin precursors *via* their 2-methoxy-2-(trifluoromethyl)phenylacetic acid (MTPA) esters, and on the asymmetric dihydroxylation of a prenyl group using AD-mix, have recently been developed in our laboratories.[2] Chiral quinoline alkaloids had been synthesised earlier, but only in racemic form or with very low ee values (< 10%).[1a]

Although many quinoline alkaloids are chiral, surprisingly few have been assigned absolute configurations or enantiopurity values (% ee). In the earlier studies, using chemically synthesised enantiopure quinoline alkaloids (assigned by unequivocal methods including X-ray crystallography),[2] we found that ten out of eleven alkaloids had opposite stereochemistry to those reported in the literature. From our current asymmetric synthesis studies of chiral alkaloids (**1C**, **2C**, **3C**, **6D** and **11F**) and coumarins (**10F** and **12F**), and the isolation from *C. ternata* (**4C**, **5D**, **8D**, **13D** and **14D**), the urgent need for a sensitive, generally applicable and rigorous stereochemical analysis method became evident. A review of the methods used earlier for stereochemical assignment of quinoline alkaloids and coumarins with structural features **B**–**E**, was thus carried out.

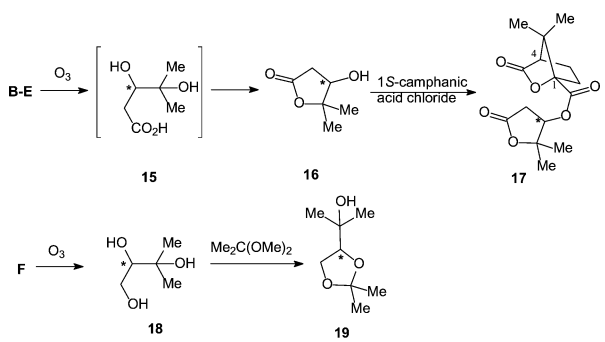


The previous approach had involved ozonolysis of the oxygenated C-prenyl compound and specific optical rotation ($[\alpha]_D$) measurements of the resulting lactone **16** (Scheme 2). However, this apparently straightforward approach, resulted in a remarkably high proportion of erroneous absolute configuration assignments for chiral quinoline alkaloids.[2] The discrepancy could have resulted from: (a) the wrong assignment of configuration of the ozonolysis product, 3-hydroxy-4,4-dimethyl-4-butyrolactone **16** (formed spontaneously from the carboxylic acid **15**), based on a lengthy stereochemical correlation sequence,[3a,b] or (b) the presence of a contaminant, presumably in small quantity and having a much greater $[\alpha]_D$ value with opposite sign to that of lactone **16**.[2] An X-ray crystallographic analysis[4] of crystalline camphanate **17**, derived from lactone **16** ($[\alpha]_D$ +11°), shows the absolute configuration to be (*R*) (Fig. 1); this provides the first unequivocal evidence in support of the original literature assignment to (+) lactone **16**.[3a,b]

We now propose an alternative approach in which μg to mg quantities of alkaloids and other natural products can be cleaved



Scheme 1



Scheme 2

† Electronic supplementary information (ESI) available: crystal data for **17**. See http://www.rsc.org/suppdata/cc/b2/b208978e/

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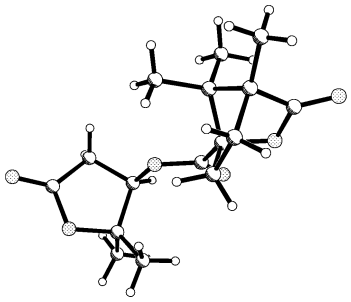

Fig. 1 X-Ray structure of camphanate **17**.

by ozonolysis and absolute configurations of the products determined by CSP–GC–MS.[5] The GC procedure used, not only effected a separation of lactone **16** from associated impurities, but also gave baseline resolution of the early (*R*) and the late (*S*) eluting enantiomers. Employment of MS as the detection system ensured that the enantiomers of lactone **16** were free from any detectable co-eluting contaminants. Ozonolysis of platydesminium methosalt **7D** (0.1 g, $[\alpha]_D$ +31°), isolated from *Skimmia japonica*, gave lactone **16** (*ca*. 50% yield) which upon analysis by the CSP-GC–MS method was found to be of (*S*) configuration; the result was in conflict with the earlier assignment[6a] of the (*R*) configuration based on ozonolysis and $[\alpha]_D$ measurement of (−)-lactone **16**.

The ozonolysis-cum-CSP-GC–MS method was next applied to a range of 1,2-diols (**1C–4C**), dihydrofurans (**5D–8D**) and a dihydropyran (**9E**) obtained either by asymmetric dihydroxylation methods or from plant extracts.

Quinoline alkaloids (+)-orixine **1C**, (−)-hydroxylunidine **3C**, (+)-isopteleflorine **6D**, and the coumarins (+)-heraclenol **10F** and (+)-oxypeucedanin hydrate **12F** and were obtained in enantiomerically enriched forms by asymmetric dihydroxylation of the corresponding alkene precursors using AD-mix and then applying the synthetic procedures reported for racemic members of the quinoline alkaloid series.[2] Included among the compounds, isolated from the leaves of *C. ternata*, were (−)-meranzin hydrate **4C**, (−)-platydesmine **5D**, (±)-balfourodinium methosalt **13D**, (±)-choisyine **14D**, (+)-evoxine **11F** and one of the new alkaloids, (−)-desmethoxychoisyine **8D**. Samples of (+)-platydesminium methosalt **7D** and (−)-geibalansine **9E** were available from earlier plant and synthetic studies.[2] The sensitivity and general applicability of the method was demonstrated by its successful application to a range of C-prenyl alkaloids and coumarins (**1C–4C**, **5D–8D** and **9E**) which in some cases were only available in sub-mg quantities (Table 1).

Ozonolysis of O-prenylated alkaloids (**11F**) and coumarins (**10F** and **12F**), following the standard conditions,[5] gave 1,2,3-trihydroxy-3-methylbutane **18**[3c] which was converted to the acetonide derivative **19** by treatment with 2,2-dimethoxypropane in the presence of a catalytic amount of *p*-TSA. Racemic (or enantioenriched) samples of acetonide **19** showed a baseline separation of the enantiomers (*S* form eluted earlier) using the same CSP-GC column and conditions as for lactone **16**.

Table 1 Optical rotations ($[\alpha]_D$), enantiomeric excess (Ee %) values and absolute configurations (Ab. Con.)

Compound	$[\alpha]_D$	Ee (%)	Ab. Con. (lit. Ab. Con.)
1C[a]	+64[c]	70	*R* (*S*)[6b]
2C[b]	+29[d]	94	*R* (*S*)[6a]
3C[a]	−2[d]	76	*S* (*R*)[7]
5D[b]	−42[d]	90	*R* (*S*)[1a,6c]
6D[a]	+18[c]	70	*S*
7D[b]	+31[e]	> 98	*S* (*R*)[6a]
8D[b]	−44[c]	64	*R*
9E[a]	−12[e]	> 98	*R*
11F[b]	+15[d]	86	*R*
4C[b]	−35[c]	92	*S* (*S*)[8a]
10F[a]	+28[c]	> 98	*R* (*R*)[8b]
12F[a]	+8[c]	49	*R* (*R*)[8b]

[a] Chemical synthesis. [b] Plant source. [c] $CHCl_3$. [d] EtOH. [e] MeOH.

From the results presented in Table 1, the following conclusions can be drawn: (i) the absolute configurations reported in the literature for (+)-orixine[6b] 1C, (+)-balfourolone[6a] 2C, (−)-hydroxylunidine[7] **3C**, (−)-platydesmine[1a,6c] **5D** and (+)-platydesminium methosalt[6a] **7D** are incorrect, (ii) literature configurations of the coumarins (−)-meranzin hydrate[8a] **4C**, (+)-heraclenol[8b] **10F** and (+)-oxypeucedanin hydrate[8b] **12F** are correct, (iii) alkaloids (+)-isopteleflorine **6D**, (−)-geibalansine **9E**, (+)-evoxine **11F** and the new alkaloid (−)-desmethoxychoisyine **8D** have been assigned absolute configurations for the first time, (iv) the hemiterpenoids **4C**, **5D**, **8D** and **11F** from *C. ternata* were found to have variable ee values (64–94%). Alkaloids **13D** and **14D** from *C. ternata* were confirmed to be racemic using the CSP-GC–MS method. This rigorous and generally applicable approach, for the determination of absolute configuration and enantiopurity, can now be extended to a wider range of oxygenated chiral hemiterpenoid natural products (alkaloids, coumarins, furochromes, rotenoids, *etc*). The reliability of the O_3-CSP-GC–MS approach has, in some cases, been successfully tested by comparison with other ee value methods (*e.g.* formation of MTPA esters of secondary alcohols)[9] and absolute configuration (*e.g.* X-ray crystallography).[2] The method should be of particular value for the analysis of natural products, containing a chiral dihydrofuran moiety and a tertiary OH group (**D**).

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4 CCDC 192859. See http://www.rsc.org/suppdata/cc/b2/b208978e/ for crystallographic data in CIF or other electronic format.

5 Ozonised oxygen was gently bubbled, through a solution of the sample (~10 mg or less) in MeOH–$CHCl_3$ (1:2, 3 cm^3), at − 20 °C, until the reaction was complete (~1 h, TLC). The concentrated residue was treated with water (2 cm^3) and the reaction mixture refluxed (0.5 h); an aq solution of. KOH (2 M, 0.5 cm^3) followed by H_2O_2 solution (30%, 0.2 cm^3) was added. It was kept at 50 °C (0.5 h), acidified (SO_2), and then left overnight at room temperature. The crude samples of lactone **16** (extracted using diethyl ether) and triol **18** (extracted using EtOAc prior to conversion to acetonide **19**) were subjected to CSP-GC–MS analysis (Supelco γ-DEX 120 capillary column, 30 m × 0.25 mm, 80 °C for 1 min and ramped at 2 °C min^{-1}. to 200 °C; mass spectrometer operated in the full scan mode, collecting ion currents between *m*/*z* 30–500 amu).

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