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Note

High-performance liquid chromatography of the *Isodon* diterpenoids

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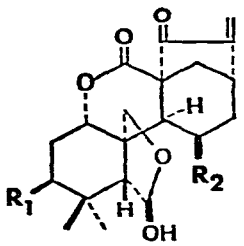
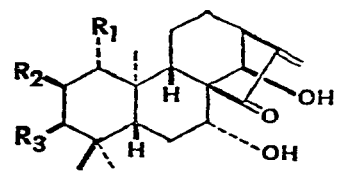
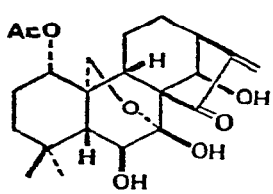
Recently, we have systematically investigated the leaf extracts of several plants of the genus *Isodon* belonging to the family *Labiatae* for their biologically active compounds[1]. This has led to the isolation of a series of highly oxygenated *ent*-kaurene and B-seco-*ent*-kaurene diterpenoids. Hitherto, all isolations were carried out by laborious conventional chromatographic techniques, but of late, high-performance liquid chromatography (HPLC) has become a method of choice for the separation of



1 R_1=OH, R_2=H
2 R_1=H, R_2=OH
3 R_1=R_2=H



5 R_1=OH, R_2=H, R_3=OAc
6 R_1=R_3=H, R_2=OH
7 R_1=OH, R_2=R_3=H



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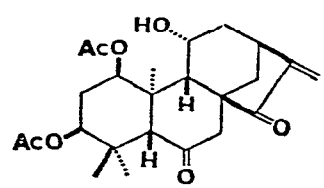

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difficultly accessible natural products. This paper describes the application of HPLC in the separation of eight highly oxygenated diterpenes of the various *Isodon* species.

METHOD AND MATERIALS

The separations were carried out on Waters Assoc. (Milford, Mass., U.S.A.) liquid chromatograph. A commercially available pre-packed μBondapak C_{18} (Waters) stainless-steel column (30 cm × 8 mm I.D.) was used. Detection was effected by UV absorption at 230 nm, with a Schoeffel GM 770 Monochromator variable-wavelength detector. Aqueous methanolic solutions (35%, v/v, of water) of the diterpene mixture (40 μg/10 μl) were injected using a Pierce Valveseal septumless injector. Analytically pure *Isodon* diterpenes from our previous studies were used in this investigation[1]. The selection of these compounds was based mainly on the availability of the samples as well as their characteristic structural types.

RESULTS AND DISCUSSION

A characteristic feature of a large number of *ent*-kaurene and B-seco-*ent*-kaurene diterpenes isolated from the plants of genus *Isodon* is the presence of the α-methylenecyclopentanone moiety. This chromophore is responsible for their UV absorption in the region 225–235 nm (ref. 2). Therefore, the presence of small amounts of the natural products bearing this functional group can easily be detected by monitoring the effluent at 230 nm. Since the products to be separated are highly polar, the reversed-phase column was chosen for the purpose.

The best separations were obtained on the μBondapak C_{18} column, with methanol–water mixtures as mobile phase. Fig. 1a shows the results obtained with four polyoxygenated diterpenes (structures 1–4), which commonly occur in *I. japonicus*, *I. trichocarpus* and *I. lasiocarpus*. By using methanol–water (55:45, v/v), baseline separation of the two diterpenes [nodosin(2) and isodocarpin(3), which differ only by an extra hydroxyl group at position C-11] was achieved, as shown in Fig. 1b. Similarly, the separation of a set of four *ent*-kaurene diterpenes structures 5–8), which are typical members of *I. kameba*, *I. umbrosus* and *I. shikokianus* var. *intermedius* was achieved by using methanol–water (60:40, v/v) as mobile phase (Fig. 2a). As with nodosin and isodocarpin, nearly baseline separation of isodomedin (5) and umbrosin A (6) was also achieved by using methanol–water (55:45, v/v) (Fig. 2b). During this work, the presence of various diterpenes could be detected in a solution containing as little as 1 μg of an individual compound.

These separation may offer an easy means for taxonomic marking of *Isodon* and other *Labiatae* plants[3]. In a forthcoming communication, we hope to report the application of this technique in the analysis of the fresh plant extract, which is currently under investigation.

CONCLUSION

HPLC on C_{18}-chemically bonded silica gel using various methanol–water mobile phases appears to offer a convenient method for the separation of kaurene and kaurene-derived diterpenes of *Isodon* possesing an α-methylenecyclopentanone func-

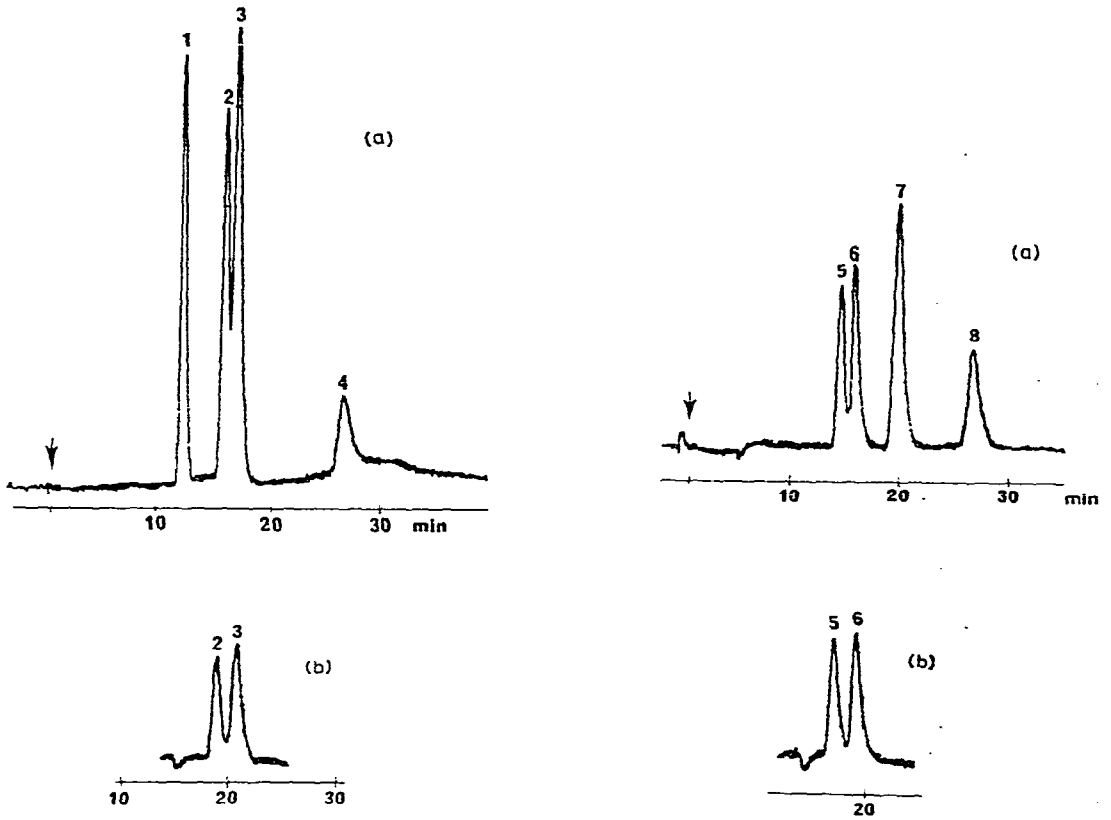

Fig. 1. (a), Separation of B-seco-*ent*-kaurene-type diterpenes (structures 1–4) on μBondapak C_{18}. Mobile phase, methanol–water (60:40, v/v) at 1.1 ml/min; detection a 230 nm. (b), Separation of nodosin (2) and isodocarpin (3). Mobile phase, methanol–water (55:45, v/v).

Fig. 2. (a), Separation of *ent*-kaurene diterpenes. Mobile phase, methanol–water (60:40, v/v) flow-rate, 1.1 ml/min. (b), Separation of isodomedin (5) and umbrosin A (6). Mobile phase, methanol–water (55:45, v/v).

tion. Therefore, it has been possible to separate differently functionalized *ent*-kaurene and B-seco-*ent*-kaurene diterpenoids on the same reversed-phase column simply by changing the composition of the mobile phase. Thus, it could be possible to expand the scope of the technique to the separation and isolation of microgram quantities of naturally occurring substances having an α-methylenecyclopentanone group.

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