oily residue (44.5 g), a part of which (30.5 g) was chromatographed on silica gel (760 g) with hexane (13 l.) and C_6H_6 (9 l.) successively. The middle fractions of the C_6H_6 eluate (480 mg) were further chromatographed on silica gel (25 g) with hexane–EtOAc (43:7). From the later fractions an amorphous solid (12 mg) was obtained, which was separated by prep. TLC with hexane–Et_2O (3:1) to give crude **1** (6.9 mg). Purification of crude **1** by prep. TLC with C_6H_6 provided crystalline **1** (2.3 mg, 0.000038%).

Laurebiphenyl (**1**). Mp 232–232.5° (from C_6H_6–hexane), $[\alpha]_D^{25}$ +15.2° ($CHCl_3$; *c* 0.092); UV λ_{max}^{MeOH} nm (ε): 208 (34300), 241 (8600, sh), 284 (5200); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600, 3480, 1610, 1565, 1495, 1155; ^1H NMR (measured at 20°): δ7.27 and 7.23 (each 1H, *s*), 6.63 (2H, *s*), 5.10 (2H, *br s*, disappeared on addition of D_2O), 2.02 and 2.00 (each 3H, *s*), 1.45 (6H, *s*), 1.28 (6H, *s*), 0.53 (4H, *m*), 0.9–2.3 (10H, *m*); ^{13}C NMR (measured at 20°): δ152.7 (*s*), 134.8 (*s*), 133.5 (*s*), 131.1 (*s*), 130.9 (*d*), 117.8 (*d*), 48.1 (*s*), 36.4 (*t*), 29.7 (*s*), 25.4 (*t*), 24.5 (*d*), 24.1 (*q*), 23.9 (*q*), 19.5 (*q*), 18.9 (*q*), 16.4 (*t*) [two signals at δ24.1 and 23.9 at 20° collapsed into one signal (δ24.0) at 60°]; MS *m/z* (rel. int.): 430 $[M]^+$ (100), 415 (36), 401 (4), 387 (4), 373 (10), 362 (10), 347 (6); HRMS *m/z* 430.2843 $[M]^+$, calc. for $C_{30}H_{38}O_2$, 430.2869.

Conversion of debromolaurinterol into **1**. A mixture of debromolaurinterol (49.0 mg) and MnO_2 (49.0 mg) in CH_2Cl_2 (2.5 ml) was stirred at room temp. for 5 min. The mixture was filtered under red. pres. Concentration of the filtrate afforded a residue, which was separated by prep. TLC with C_6H_6 to give **1** (10.8 mg, 21%) and unreacted debromolaurinterol (35.2 mg, 72%). Recrystallization from C_6H_6–hexane yielded **1** as colourless crystals, mp 233–233.5°; $[\alpha]_D^{25}$ +16.6° ($CHCl_3$; *c* 1.43). The synthetic **1** was proved to be identical with natural **1** by comparison of the spectral (UV, IR, ^1H NMR, ^{13}C NMR and MS) data and chromatographic behaviour.

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REFERENCES

1. Moore, R. E. (1978) in *Marine Natural Products* (Scheuer, P. J., ed.) Vol. 1, p. 43. Academic Press, New York.
2. Martin, J. D. and Darias, J. (1978) in *Marine Natural Products* (Scheuer, P. J., ed.) Vol. 1, p. 125. Academic Press, New York.
3. Erickson, K. L. (1983) in *Marine Natural Products* (Scheuer, P. J., ed.) Vol. 5, p. 131. Academic Press, New York.
4. Shizuri, Y., Yamada, A. and Yamada, K. (1984) *Phytochemistry* **23**, 2672.
5. Caccamese, S. and Rinehart, K. L. (1978) in *Drugs and Food from the Sea* (Kaul, P. N. and Sinderman, C. J., eds) p. 187. University of Oklahoma Press, Norman.
6. Irie, T., Suzuki, M., Kurosawa, E. and Masamune, T. (1966) *Tetrahedron Letters* 1837.
7. Irie, T., Suzuki, M., Kurosawa, E. and Masamune, T. (1970) *Tetrahedron* **26**, 3271.
8. Cameron, A. F., Ferguson, G. and Robertson, J. M. (1967) *J. Chem. Soc. Chem. Commun.* 271.
9. Cameron, A. F., Ferguson, G. and Robertson, J. M. (1969) *J. Chem. Soc. B* 692.

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TERPENOIDS FROM *SALVIA PALAESTINA*

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Key Word Index—*Salvia palaestina*; Labiatae; terpenoids; sclareol; antibacterial activity.

Abstract—Six known terpenoids: vergatic acid, ursolic acid, crataegolic acid, lupane-3β-,11α,20-triol, sclareol and sitosteryl 3β-glucoside were isolated from the leaves of *Salvia palaestina* and were identified by spectral data. Among the compounds, sclareol showed high activity against *Staphylococcus aureus*, *S. epidermis*, *Escherichia coli*, *Proteus vulgaris* and *Pseudomonas aeruginosa*, while the triterpenoids were not tested due to solubility problems.

In a previous study with the benzene extract of *Salvia palaestina* Bentham we described the identification of 16 flavonoids and antibacterial activity of cirsimaritin [1]. A further investigation of the same extract has led to the isolation of terpenic compounds, vergatic acid [2], ursolic acid [3], crataegolic acid [4], sclareol [5], sitosteryl 3β-glucoside [6] and lupane-3β,11,20-triol [7]. One of the terpenoids, sclareol, showed a high antibacterial activity against standard test strains of *Staphylococcus aureus*, *S. epidermis*, *Escherichia coli*, *Proteus vulgaris* and

Table 1. Antibacterial activity of sclareol with standard bacterial strains

Bacteria	*S. aureus* ATCC 6538		*S. epidermidis* ATCC 12228		*E. coli* ATCC 8739		*P. vulgaris* ATCC 8427		*P. aeruginosa* ATCC 1539	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Sclareol	15.6	31.25	15.6	31.25	62.5	125	62.5	125	31.25	250

Pseudomonas aeruginosa. Because of solubility problems, the other compounds were not tested.

Spectral data of the first three compounds indicated the presence of olean-12-en-20-oic acids and standard sample comparisons (IR, TLC) proved that these were vergatic acid, ursolic acid and crataegolic acid. The ^{1}H NMR, ^{13}C NMR and MS data for the fourth compound indicated that it was a diterpene alcohol, sclareol. Hydrolytic and spectral data for the fifth compound, as well as standard sample comparison (IR, TLC) proved its identity as sitosteryl 3β-glucoside.

According to the mass spectrum of the last compound (**1**) the highest peak at *m/z* 442 corresponded to an apparent molecular formula $C_{30}H_{50}O_2$. Acetylation of **1** yielded a diacetate, consistent with the presence of two hydroxyl groups. The ^{1}H NMR spectrum of **1** showed eight methyl singlets at δ0.78, 0.96, 1.01, 1.08, 1.15, 1.26 and 1.28 and there were no vinylic methyls or vinylic hydrogens present. The chemical shift of a proton geminal to one of the hydroxyl groups at δ3.2 (*t*, J = 6 and 11 Hz) indicated it to be in a C-3 equatorial position while the other proton geminal to the second hydroxyl group was at δ3.95 (*ddd*, J = 5, 12 and 11 Hz) from which it is situated between a methylene and a methine group and should be axial. Since there was no vinylic proton in the ^{1}H NMR spectrum, there should either be a tetrasubstituted double bond or no double bond in the molecule. A literature search failed to reveal any known compound with these structural features fitting the apparent formula, but the data appeared to be fully consistent with those published for lupane-3β,11α,20-triol ($C_{30}H_{52}O_3$), which did not yield a molecular ion in its EI mass spectrum, but showed an $[M - H_2O]^+$ peak at *m/z* 442 [7]. Direct comparison of the 400 MHz ^{1}H NMR and mass spectra of compound **1** with those of an authentic sample of lupane-3β,11α,20-triol showed them to be identical.

EXPERIMENTAL

Plant material. The plant material used in the previous study [1] was used again here.

Identification of terpenoids. A C_6H_6 extract of the plant material was fractionated on a silica gel column (4 × 50 cm). The terpenoids were mixed with small amounts of flavonoids and these were separated on Sephadex LH-20 columns by eluting with EtOH. The first fractions yielded single compounds, eluted in the following order: ursolic acid (30 mg), vergatic acid (10 mg), sclareol (500 mg), crataegolic acid (20 mg), lupane-3β,11α,20-triol (15 mg), sitosteryl 3β-glucoside (50 mg).

Antibacterial activity. Details of the disc-diffusion method are given in the previous paper [1]. Sclareol showed high activity against the same standard strains (Table 1); the other compounds were not tested because of their poor solubility in EtOH.

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REFERENCES

1. Miski, M., Ulubelen, A., Johansson, C. and Mabry, T. J. (1983) *J. Nat. Prod.* **46**, 874.
2. Ulubelen, A. and Ayanoglu, E. (1975) *Phytochemistry* **14**, 820.
3. Brieskorn, C. H., Klinger, H. and Polonius, W. (1961) *Arch. Pharm.* **294**, 389.
4. Ulubelen, A., Miski, M. and Mabry, T. J. (1981) *J. Nat. Prod.* **44**, 119.
5. Nicholas, H. J. (1958) *J. Am. Pharm. Ass. Sci. Ed.* **47**, 731.
6. Ulubelen, A. and Uygur, I. (1976) *Planta Med.* **29**, 318.
7. Garcia-Alvarez, M. C., Savona, G. and Rodriguez, B. (1981) *Phytochemistry* **20**, 481.