extr). The extracts were dried (Na_2SO_4), the solvent removed in a rotary evaporator in the dark, at room temp. and the residue subjected to silica gel CC using petrol–Et_2O mixtures containing increasing amounts of Et_2O (assayed by UV). The fractions containing polyacetylenes were successively rechromatographed on a Chromatotron (gradient elution petrol–Et_2O; 5 ml/min) and by PLC (continuous elution) at 0°, in the dark, providing pure compounds.

Pterocaulon allopecuroides (Lam) DC. Plants were collected in February 1986 in Campinas (SP-Brasil). A voucher specimen is deposited in the Herbarium under No. 2706 (UEC). Fresh roots (690 g) provided **1** (58 mg), **3** (39 mg), **4** (20 mg), **6** (9 mg) and **7** (17 mg).

P. balansae Chodat. Plants were collected in August 1986 in Campinas. A voucher specimen is deposited in the Herbarium under No. 2709 (UEC). Fresh roots (1800 g) afforded **1** (120 mg), **2** (13 mg), **3** (92 mg), **5** (9 mg), **6** (13 mg) and **8** (14 mg).

P. lanatum O. Kuntze. Plants were collected in March 1987 in Americana (SP). A voucher specimen is deposited in the Herbarium under No. 2710 (UEC). Fresh roots (3700 g) provided **1** (104 mg), **3** (160 mg), **5** (11 mg), **6** (22 mg) and **8** (15 mg).

P. rugosum (Vahl.) Malme. Plants were collected in Ibitinga, near S. Carlos (SP). A voucher specimen is deposited in Herbarium under No. 25192 (UEC). Fresh roots (1300 g) provided **1** (81 mg), **3** (50 mg), **5** (10 mg), **6** (7 mg) and **8** (23 mg).

Tridec-1,2-dimethoxy-3,5,7,9,11-pentyne (**2**). Very unstable liquid; UV $\lambda_{max}^{Et_2O}$ nm (ε): 237.5 (104 000). 250 (226 000) and 264 (334 000); ^{1}H NMR (80 MHz, CCl_4): δ2.02 (*s*, 3H), 3.22–3.31 (*m*, 3H), 3.70 (*s*, 6H).

REFERENCES

1. Johns, R. S., Lamberton, J. A., Price, J. R. and Sioumis, A. A. (1968) *Aust. J. Chem.* **21**, 3079.
2. Magalhães, A. F., Magalhães, E. G., Leitão Filho, H. F., Frighetto, R. T. S. and Barros, M. S. G. (1981) *Phytochemistry* **20**, 1369.
3. Debenedetti, S. L., Ferraro, G. E. and Coussio, J. D. (1981) *Planta Med.* **42**, 97.
4. Bohlmann, F., Abraham, W.-R., King, R. M. and Robinson, H. (1981) *Phytochemistry* **20**, 825.
5. Bohlmann, F., Burkhardt, T. and Zdero, C. (1973) *Naturally Occurring Acetylenes*. Academic Press, London.
6. Stahl, E. (1964) *Angew. Chem. Int. Ed. Engl.* **3**, 784.
7. Hostettmann, K., Hostettmann-Kaldas, M. and Sticher, O. (1980) *J. Chromatog.* **9**, 366.
8. Bohlmann, F. and Bresinsky, E. (1967) *Chem. Ber.* **100**, 1209.
9. Bohlmann, F., Kleine, K.-M. and Arndt, C. (1964) *Chem. Ber.* **97**, 2125.
10. Schulte, K. E., Reisch, J. and Hörner, L. (1960) *Angew. Chem.* **72**. 920.
11. Bohlmann, F., Grenz, M. Wotschokowsky, M. and Berger, E. (1967) *Chem. Ber.* **100**, 2518.
12. Bohlmann, F., Arndt, C., Kleine, K.-M. and Bornowski, H. (1965) *Chem. Ber.* **98**. 155.
13. Bohlmann, F., Blaszkiewicz, P. and Bresinsky, E. (1968). *Chem. Ber.* **101**, 4163.
14. Bohlmann, F. and Zdero, C. (1970) *Chem. Ber.* **103**, 834.
15. Bohlmann, F. and Zdero, C. (1977) *Phytochemistry* **16**, 1832.

Phytochemistry, Vol. 28, No. 9, pp. 2499–2501, 1989.
Printed in Great Britain.

0031-9422/89 $3.00 + 0.00
© 1989 Maxwell Pergamon Macmillan plc.

A DIHYDROXYCYCLOPENTADIENONE AND OTHER CONSTITUENTS FROM THE SEEDS OF *TRIFOLIUM REPENS*

MUNEHIRO NAKATANI,* MIKIO TAJIRI, TOSHIHIRO KAGA, RYOKO FUKAMI, YOSHIHIRO TAKADA and TSUNAO HASE

Department of Chemistry, Faculty of Science, Kagoshima University, 1-21-35 Korimoto, Kagoshima 890, Japan

(*Received in revised form* 9 *January* 1989)

Key Word Index—*Trifolium repens*; Leguminosae; white clover; fatty acids; sterols; triterpene; flavonols; (2*R*,3*R*)-butanediol; 3-hydroxy-2-methyl-4-pyrone; 2,3-dihydroxy-2,4-cyclopentadien-1-one.

Abstract—A new natural substance, 2,3-dihydroxy-2,4-cyclopentadien-1-one, was isolated from the seeds of white clover along with many known compounds.

INTRODUCTION

There are a number of papers on the constituents of the seeds of *Trifolium repens* L. [1–4], in some of which it is reported that myricetin and some condensed tannins are toxic to *Rhizobium* bacteria [2, 4]. In the course of our studies on biologically active natural products, we re-examined the constituents of the seeds. Although no new active compounds were obtained, a new natural substance, 2,3-dihydroxy-2,4-cyclopentadien-1-one (**1**), was isolated along with many known compounds, of which (2*R*,3*R*)-butanediol (**2**) and 3-hydroxy-2-methyl-4-pyrone were isolated for the first time from clover.

1 R = H
3 R = Me

1a

RESULTS AND DISCUSSION

The seeds were extracted with methanol followed by hot water. The methanol extract was fractionated with hexane, ether and ethyl acetate. The hexane extract afforded seven saturated long-chain fatty acids, linoleic acid and their methyl esters, β- and γ-sitosterol, stigmastanol and β-amyrin. From the ether extract, 3-hydroxy-2-methyl-4-pyrone was isolated. The ethyl acetate extract afforded three flavonols, quercetin, myricetin and kaempferol. A new natural compound (**1**) was obtained along with (2*R*,3*R*)-butanediol (**2**) and succinic acid from the water soluble extract.

Compound **1**, $C_5H_4O_3$, mp 208°(dec), showed a blue coloration with ferric chloride reagent. Its IR spectrum exhibited hydroxyl (3300–3000 cm^{-1}) and carbonyl (1700–1660 cm^{-1}) signals and the UV spectrum showed two absorption max at 218 (ε 2440) and 258 nm (ε 3400). Because of the solubility of **1** in all common organic NMR solvents, a dimethyl ether **3** prepared by treating of **1** with diazomethane was used for NMR studies. The ^{1}H NMR spectrum of **3** only showed two methoxy proton signals at δ 3.40 and 3.46, and conjugated olefinic proton signals at δ 5.86 and 7.31 coupling ($J = 8$ Hz) with each other. Two UV absorptions were also observed at 217 and 263 nm. These results suggested that the structure of **2** was represented as shown and the UV data of **1** and **2** elucidated almost complete predominance of the intra-molecular bonded cisoid form in **1**. In this steric situation, the β-diketone form **1a** is less stable. Thus **1** is 2,3-dihydroxy-2,4-cyclopentadien-1-one.

The stereochemistry of 2,3-butanediol (**2**) was clarified as 2*R* and 3*R* by a CD study using nickel acetylacetonate [5]. Strong split Cotton effects were observed at 318(Δ_ε +19) and 297 nm(Δ_ε −9) to reveal the (−)-chirality of the 2,3-diol group in the stable conformation.

Myricetin, 3-hydroxy-2-methyl-4-pyrone and (+)-2,3-butanediol (**2**) have been reported as antibacterial [2, 6] and antifungal [7] substances, respectively, but both they and compound **1** showed little activity against the bacteria and fungi used in our assays.

EXPERIMENTAL

Mps: uncorr. TLC was performed on Kieselgel 60 F_{256} precoated silica gel plates (Merck) and HPLC was carried out on a semiprep C_{18} column (Waters).

Extraction and isolation. The seeds (5 kg) of *T. repense* were extracted with MeOH(3 × 5 l). After concn, H_2O(1 l) was added and the soln was extracted with hexane, Et_2O and EtOAc. The hexane extract (9.3 g) was chromatographed on silica gel using a C_6H_6–hexane solvent system to give three fractions. Fraction1 (1.1 g) was a mixture of fatty acid methyl esters, which was analysed by GC-MS to show six components: methyl tetradecanoate, pentadecanoate, hexadecanoate, linoleate (main component) [8] and triacontanoate. Fraction 2 (750 mg) was purified by rechromatography, TLC separation and recrystallization to give β-sitosterol (72 mg), a mixture of β-sitosterol and stigmastanol (320 mg) [9], clionasterol (6 mg) [10] and β-amyrin (40 mg). Fraction 3 (470 mg), being a mixture of fatty acids, was methylated with CH_2N_2 to give methyl esters, which were analysed by GC-MS. The eight components present were: methyl tetradecanoate, pentadecanoate, hexadecanoate, heptadecanoate, linoleate, octadecanoate, eicosanoate and tetraeicosanoate.

The Et_2O extract (3.2 g) was chromatographed on silica gel with Et_2O and then each of the crystalline fractions was rechromatographed to give 3-hydroxy-2-methyl-4-pyrone (21 mg) and a mixture of flavonols (45 mg). The EtOAc extract (4.5 g) afforded three yellow crystalline fractions on DCCC using $CHCl_3$–MeOH–H_2O (7:13:8) as solvent system in the ascending mode. Each compound was purified by HPLC using H_2O–MeOH and crystallized to give myricetin (25 mg), quercetin (85 mg) and kaempferol (14 mg).

After extraction with MeOH, the seeds were extracted with boiling H_2O (3 × 10 l) and, after concn to 1 l under red. pres., the H_2O extract was extracted with EtOAc to give an extract (11 g). EtOAc was added to the extract to ppt. a powder (750 mg), which was chromatographed to give **1** (335 mg) and succinic acid (38 mg). The EtOAc soln was then chromatographed to give (2*R*,3*R*)-butanediol (**2**)(1.1 g). All the known compounds were identified by direct comparison with authentic samples or from published data.

2,3-Dihydroxy-2,4-cyclopentadien-1-one (**1**). White powder, mp 208° (dec); EIMS *m/z*: 112[M]$^+$; UV λ_{max}^{MeOH} nm (ε): 218 (2440), 258 (3400); IR ν_{max}^{Nujol} cm^{-1}: 3300–3000, 1700–1660.

Dimethyl ether **3**. Compound **1** (50 mg) was methylated with CH_2N_2 in MeOH to give the methyl ether **3** (43 mg), mp 123–125°; EIMS *m/z*: 140 [M]$^+$; UV λ_{max}^{MeOH} nm (ε): 217 (2300), 263 (3300); IR ν_{max}^{Nujol} cm^{-1}: 1710–1670; ^{1}H NMR($CDCl_3$): δ 3.40 (3H, *s*), 3.46 (3H, *s*), 5.86 (1H, *d*, $J = 8$ Hz), 7.31 (1H, *d*, $J = 8$ Hz). (Found C, 60.02; H, 5.77; $C_7H_8O_3$ requires C, 59.99, H, 5.75%).

(2R,3R)-Butanediol (2). Oil, $[\alpha]_D$ +11°(MeOH; *c* 1.1); CD[$CHCl_3$ + Ni(acac)$_2$] nm: $\Delta_{\varepsilon 297}$ −9, $\Delta_{\varepsilon 318}$ +19.

Antimicrobial activity. The effects of compounds **1** and **2**, myricetin and 3-hydroxy-2-methyl-4-pyrone on the growth of microorganisms were tested by a broth dilution method [12] by Dr M. Taniguchi (Osaka City University). They showed no activity at 100 μg/ml concn. The test organisms used were the bacteria: *S. aureus*, *B. subtilis*, *E. coli* and *Ps. auruginosa*; the fungi: *Mucor mucedo*, *Rh. chinensis* and *Asp. niger*; and the yeasts: *S. cerevisiae*, *C. utilis* and *Schiz. pombe*.

Acknowledgements—We are grateful to Dr M. Taniguchi (Osaka City University) for the antimicrobial test and Dr K. Shibata (Osaka City University) for the elemental analysis. Our thanks are also due to Dr H. Naoki (Suntory Institute for Bioorganic Research) for CI and SIMS spectra.

REFERENCES

1. Horvei, K. F. and Wickstroem, A. (1964) *Acta Chem. Scand.* **18**, 833.
2. Fottrell, P. E., O'Connor, S. and Masterson, C. L. (1964) *Ir. J. Agric.* **3**, 246.
3. Beveridge, R. J., Ford, C. W. and Richard, G. N. (1977) *Aust. J. Chem.* **30**, 1583.

4. Young, H. and Paterson, V. J. (1980) *Phytochemistry* **19**, 159.
5. Dillon, J. and Nakanishi, K. (1974) *J. Am. Chem. Soc.* **96**, 4059.
6. Morris, J. A., Khettry, A. and Seitz, E. W. (1979) *J. Am. Oil Chem. Soc.* **56**, 595.
7. Rosenlerg, S. (1962) *Chem. Abs.* **56**, 73901.
8. Stenhagen, E., Abrahamsson, S. and Mclafferty, F. W. (1974) *Registry of Mass Spectral Data*, p. 1833. Wiley, New York.
9. Iida, T., Tamura, T. and Matsumoto, T. (1981) *Phytochemistry* **20**, 857.
10. Stenhagen, E., Abrahamsson, S. and Mclafferty, F. W. (1974) *Registry of Mass Spectral Data*, p. 2660. Wiley, New York.
11. Buttery, R. G., Komm, J. A. and Ling, L. C. (1984) *J. Agric. Food Chem.* **32**, 254.
12. Taniguchi, M. and Satomura, Y. (1972) *Agric. Biol. Chem.* **36**, 2169.

Phytochemistry, Vol. 28, No. 9, pp. 2501–2502, 1989.
Printed in Great Britain.

0031-9422/89 $3.00 + 0.00
Maxwell Pergamon Macmillan plc.

THE ISOLATION OF A GUAIANE SESQUITERPENE FROM FRUIT BODIES OF *LACTARIUS SANGUIFLUUS*

OLOV STERNER, OLA BERGENDORFF and FABIENNE BOCCHIO

Department of Organic Chemistry, Lund Institute of Technology, P.O.B. 124, S-221 00 Lund, Sweden

(*Received* 5 *December* 1988)

Key Word Index—*Lactarius sanguifluus*; Agaricaceae; mushroom; guaiane sesquiterpene; sangol.

Abstract—The isolation and structure elucidation of a novel guaiane alcohol, formed together with related aldehydes and alcohols from fatty acid ester precursors in injured fruit bodies of the Basidiomycete *Lactarius sanguifluus*, is reported.

The fruit bodies of the *Lactarius* species belonging to the Dapetes Fr. section (Basidiomycotina subdivision of Fungi) have yielded a series of sesquiterpenoids with a guaiane skeleton, which appear to be enzymatically converted into each other in injured fruit bodies [1, 2]. The fruit bodies of *Lactarius deliciosus* Fr. and *L. deterrimus* Gröger for instance, originally contain the stearic acid ester **1a** (together with small amounts of the corresponding linoleic acid ester [1]) as the only sesquiterpenoid. If the fruit bodies of these species are injured, the ester **1a** is converted to the aldehyde **1b**, to the free alcohol **1c**, as well as to lactaroviolin **2b** and deterrol **2c** [1]. The stearic ester of the latter, i.e. the ester **2a**, has never been detected in the fruit bodies of *L. deliciosus* and *L. deterrimus*, but was instead isolated from the fruit bodies of *L. indigo* (Schw.) Fr. [3] together with lactaroviolin **2b**. Recently, the aldehyde **3b** was isolated from the fruit bodies of *L. sanguifluus* Paulet ex Fr. [4], a species that previously has yielded the ester **1a**, the alcohol **1c**, lactaroviolin **2b**, as well as an unidentified azulene called 'lipophiles lactaroviolin' [2]. It was not clear whether the aldehyde **3b** is present as such in the fruit bodies or if it is formed enzymatically from a precursor, and in order to establish this we performed an investigation of both the initial sesquiterpenoid contents of the fruit bodies of *L. sanguifluus*, as well as of the nature of any new compounds formed in injured specimens.

In accordance with the situation observed in the fruit bodies of *L. deliciosus* and *L. deterrimus*, no traces of the free sesquiterpenes **1b**, **1c**, **2b**, **2c** or **3b** could be detected by TLC analysis of hexane extracts of young and undamaged fruit bodies of *L. sanguifluus* (extracted directly after collection). Instead, the presence of the ester **1a** could be demonstrated by comparison of ^{1}H NMR and TLC data with an original sample isolated from *L. deliciosus*. Besides the yellow ester **1a**, the presence of an equally nonpolar but red compound was indicated, but due to the instability of the compounds, the limited amounts available, and their similar chromatographic properties, it was not possible to separate the two completely. However, inspection of the ^{1}H NMR spectrum of a purified fraction of the unidentified red compound, and comparison of this with those of the other sesquiterpenoids discussed here, strongly suggest that it is the ester **3a**. The only difference (except for the signals of the fatty acid protons) compared to the ^{1}H NMR spectrum of the new alcohol **3c** (for which we propose the name sangol, structure discussed below), is that the signal for H_2-15 was shifted downfield from δ4.58 in the spectrum of sangol **3c**, to δ5.04 in the spectrum of the compound believed to be ester **3a**. We believe that this compound is identitical to 'lipophiles lactaroviolin' [2]. Sangol **3c** was isolated by rapid silica gel chromatography from an ethyl acetate extract of fruit bodies of *L. sanguifluus* that had