Therefore, when *Gibberella fujikuroi* was incubated with **1b** at a concentration of 40 mg/l., GA_3 production was inhibited from mevalonate as well as from acetate. This result is in contrast to the analogous situation for the biosynthesis of cholesterol from these precursors which has been reported [1] for several animals.

EXPERIMENTAL

Preliminary expts were undertaken using incubations of compactin or its Na salt (**1b**) (formed by stirring compactin in 0.1 M NaOH for 2 hr at 50°) in 250 ml shake flask cultures of *Gibberella fujikuroi*, strain GF-1a. Analysis by GC (of Me esters, TMSi ethers) of the metabolites produced after 5 days showed the following. (1) Neither **1a** nor **1b** affected qualitatively the metabolites formed: the major ones being GA_3, GA_{13} and fujenal diacid in the ratio 20:6:9. (2) Metabolite levels were decreased by up to 90% by addition of the Na salt **1b**. (3) Compound **1b** was much more effective in reducing metabolite levels than **1a**. (4) The optimum dose of **1b** appeared to be *ca* 40 mg/l.

Consequently, incorporations of sodium [2-^{14}C]acetate and [2-^{14}C]mevalonolactone into GA_3 were measured at 40 mg/l. of **1b**, as follows.

Aq. sodium [2-^{14}C]acetate (138 kBq) was added to a 250 ml shake flask containing sterilized 0% ICI medium [2] (50 ml) and **1b** (2.0 mg). Freshly Buchner-filtered and H_2O-washed mycelium from a 4-day-old culture of *Gibberella fujikuroi*, strain GF-1a, was then added. As a control, the above expt was repeated but without the addition of **1b**. The incubations with added [2-^{14}C]mevalonolactone (EtOH soln of 3-(*RS*) isomers, 62.5 kBq/250 ml shake flask) were performed under identical conditions. All incubations were done in triplicate (total of 12). After shaking for 5 days at 200 orbits/min the mycelia were filtered off and 100 mg cold GA_3 added to each filtrate. The filtrates were then adjusted to pH 2 with 2N HCl, extracted with EtOAc (3 × 50 ml), washed with H_2O (50 ml), concd *in vacuo*, and chromatographed by prep. TLC (Merck Kieselgel 60 HF_{254}; 0.8 mm thick; EtOAc–$CHCl_3$–HOAc, 15:5:1), eluting the band at R_f 0.45 using H_2O-satd EtOAc. The resultant GA_3 was then crystallized from Me_2CO–petrol to constant specific radioactivity. The results are indicated in Table 1.

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TRITERPENOIDS FROM *HYPTIS SUAVEOLENS* ROOTS

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Key Word Index—*Hyptis suaveolens*; Labiatae; triterpenoids; α-amyrin; β-amyrin; 3β-hydroxylup-12-en-28-oic acid.

Abstract—A new natural triterpenoid, 3β-hydroxylup-12-en-28-oic acid, has been isolated from the roots of *Hyptis suaveolens* in addition to α- and β-amyrin.

INTRODUCTION

In continuation of our earlier work [1–3] we have isolated three more triterpenoids from the roots of *Hyptis suaveolens*, and their structural elucidation is described in the present communication.

RESULTS AND DISCUSSION

The first compound was shown to be α-amyrin by mp, IR and colour tests [4–7]. This was confirmed by co-TLC with an authentic sample. Similarly the second compound proved to be β-amyrin.

The third compound, mp 288–289° (alcohol), $[\alpha]_D^{30}$ +12 (pyridine), gave colour reactions [4–7] which indicated that it is unsaturated. Elemental analyses and MW (456) determination by mass spectrometry gave the molecular formula $C_{30}H_{48}O_3$. The IR spectrum showed hydroxyl (3440 cm^{-1}), carboxyl (1690 cm^{-1}), unsaturation (1645 cm^{-1}) and gem-dimethyl (1380 and 1370 cm^{-1}) functions in the molecule. Also, the –COH stretching bands at 1035 and 1025 cm^{-1} showed that the hydroxyl group is equatorial and is present at the C-3 position of an A/B *trans*-triterpenoid [8].

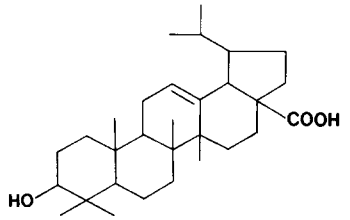
In the ^{1}H NMR spectrum, singlets at δ 0.66 (6H, *s*), 0.76 (3H, *s*), 0.90 (3H, *s*) and 1.04 (3H, *s*) demonstrated five tertiary methyl groups [9]. Two doublets appearing at

δ 1.12 (3H, *d*, $J = 6.5$ Hz) and 1.20 (3H, *d*, $J = 6.5$ Hz) showed two secondary methyl groups. Further, the spectrum displayed one proton singlet at δ 4.63 which could be attributed to a hydroxyl proton. A single proton resonating as a triplet was centred at δ 5.10 (1H, *t*, $J = 2.5$ Hz) which is a typical feature of a ring olefinic proton. The appearance of another one-proton triplet centred at δ 4.47 (1H, *t*, $J = 7.0$ Hz) showed the presence of a hydroxymethine adjacent to a single methylene group in the molecule [10].

Examination of the mass spectrum revealed the presence of fragment ions at *m/z* 456 $[M]^+$, 413, 411, 248 (base peak), 208, 207, 205, 203, 191, 190, 189 and 160. Appearance of peaks at *m/z* 248 and 208 is accountable on the basis of the retro-Diels–Alder fragmentation pattern [11, 12] which has been recognized as a characteristic feature of the mass spectra of Δ^{12}-unsaturated triterpenoids [13]. Also, the formation of these two fragment ions required the placement of a hydroxyl group at C-3 and a lone carboxyl group to either of the rings C, D or E. A peak at *m/z* 205 is attributable to the fragment ion formed by the loss of an isopropyl unit from the fragment corresponding to the base peak. It further loses a carboxyl species and gives an ion radical appearing at *m/z* 160.

Perusal of IR, NMR and mass spectral data, shows that this compound is a member of the lup-12-ene series of triterpenoids. The fragment corresponding to *m/z* 203 is of a very high intensity, next only to the base peak, indicative of its easy formation. This suggests loss of a carboxyl group from the ion at *m/z* 248 with the carboxyl group situated in a considerably hindered position. Possible positions in rings C, D and E could only be as C-27 or C-28. Of the two possibilities, the assignment of a carboxyl group as C-27 is ruled out on the basis of the following arguments.

During chemical investigation of pentacyclic triterpene acids. Nagai *et al.* [14] observed that methylation of C-27 acids with diazomethane required *ca* 15 hr, whereas Ogura *et al.* [15] have reported that a similar reaction with C-28 acids is accomplished within 10 min. We have found that the acetate derivative of the compound under discussion, on being treated with diazomethane, is methylated within 10 min. In view of this, it can be concluded that the carboxyl group is located as C-28 and not as C-27. Therefore, the structure of this compound is assigned as 3β-hydroxylup-12-en-28-oic acid (**1**).



1

The monoacetate [mp 293–295°, IR ν_{max}^{KBr} cm^{-1}: 1737, 1245, (O–CO–Me), 1690 (carboxyl carbonyl) and 1645 (unsaturation)] on treatment with ethereal diazomethane gave the monomethyl ester acetate, mp 215–216°, IR ν_{max}^{KBr} cm^{-1}: 1739, 1250 (ester carbonyl) and 1640 (unsaturation). Allison *et al.* [16], during the course of a chemical reaction, incidently obtained 3β-hydroxylup-12-en-28-oic acid and prepared its acetate and methyl ester acetate derivatives. The mp (288–289°) and optical rotation (+12° in pyridine) of the isolated natural product were found to be in close agreement with the mp (287–289°) and optical rotation (+11° in pyridine) of 3β-hydroxylup-12-en-28-oic acid, synthesized by them. The identification of the natural product as 3β-hydroxylup-12-en-28-oic acid was finally confirmed by comparing the mps of its acetate and monomethyl ester acetate derivatives with those of the synthetic products.

EXPERIMENTAL

All mps are uncorr. Optical rotations were measured in $CHCl_3$ or pyridine. Si gel G was used for TLC and the detections were made by heating the plates in an oven after spraying with 10% H_2SO_4.

Extraction and isolation of compounds. Air-dried and powdered roots (5 kg) of *H. suaveolens* were extracted with hot C_6H_6 and the extract was concd *in vacuo*. The extract (70 g) was chromatographed over Si gel, eluting with hexane, hexane–C_6H_6 (3:1, 1:1, 1:3), C_6H_6, C_6H_6–EtOAc (3:1, 1:1, 1:3) and EtOAc in succession.

α-Amyrin. C_6H_6 eluate gave a colourless crude solid which on fractional crystallization from alcohol yielded colourless needles (120 mg), mp 185–186°, $[\alpha]_D^{25}$ +84° ($CHCl_3$); IR ν_{max}^{KBr} cm^{-1}: 3380, 1620, 1380, 1365, 1040, 1027 and 995. It gave a positive L–B, Noller's and TNM tests (acetate mp 223–225°).

β-Amyrin. C_6H_6 eluate further gave a compound which crystallized from alcohol into colourless crystals (130 mg), mp 195–196°, $[\alpha]_D^{25}$ +88° ($CHCl_3$); IR ν_{max}^{KBr} cm^{-1}: 3350, 1625, 1380, 1360, 1035 and 995. It responded positively to the tests for triterpenoids (acetate 233.)

3β-Hydroxylup-12-en-28-oic acid. Obtained from the C_6H_6–EtOAc (1:3) fraction and gave (EtOH) crystals (310 mg), mp 288–289°, $[\alpha]_D^{30}$ +12° (pyridine); IR ν_{max}^{KBr} cm^{-1}: 3440, 1690, 1645, 1380, 1370, 1035, 1025 and 995; ^{1}H NMR (DMSO-d_6): δ 0.66 (6H, *s*), 0.76 (3H, *s*), 0.90 (3H, *s*), 1.04 (3H, *s*), 1.12 (3H, *d*, $J = 6.5$ Hz), 1.20 (3H, *d*, $J = 6.5$ Hz), 4.47 (1H, *t*, $J = 7.0$ Hz), 4.63 (1H, *s*), 5.10 (1H, *t*, $J = 2.5$ Hz); MS *m/z* 456 $[M]^+$, 439, 438, 413, 412, 411, 395, 248 (base peak), 247, 220, 219, 208, 207, 205, 203, 191, 190, 189, 160 and 145.

Methyl-3β-acetoxylup-12-en-28-oate. The acetate (mp 293–295°, IR ν_{max}^{KBr} cm^{-1}: 1737, 1690, 1645, 1380, 1370, 1245, 1030, 980 and 920) prepared with Ac_2O–pyridine was methylated in Et_2O with CH_2N_2 at room temp. for 10 min. The mixture was dried under red. pres. to give a solid mass which on repeated crystallization (MeOH) yielded colourless shining crystals (25 mg), mp 215–216°, IR ν_{max}^{KBr} cm^{-1}: 1739, 1640, 1380, 1370, 1250, 1150, 1020, 900 and 880.

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HOPENOL-B, A TRITERPENE ALCOHOL FROM *EUPHORBIA SUPINA*

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Key Word Index—*Euphorbia supina*; Euphorbiaceae; triterpenes; motiol; hopenol-b.

Abstract—In addition to the known triterpene motiol, hopenol-b (hop-22(29)-en-3β-ol) was isolated from the whole herb of *Euphorbia supina*. It has not been found previously in nature, though it has been prepared by both synthetic and biosynthetic methods.

INTRODUCTION

Euphorbia supina Rafin. has been reported to contain octacosanol, sitosterol and an unidentified triterpene alcohol [1]. Recent investigation on the neutral benzene extract of this plant demonstrated the presence of fern-8-en-3β-ol and supinenolone-C (3β-hydroxyfern-8-en-7,11-dione) [2].

In this paper, the isolation of motiol and a new triterpene alcohol is described.

RESULTS AND DISCUSSION

Continuous CC of the neutral benzene extract of the air-dried whole herb afforded, after the elution of fern-8-en-3β-ol, a solid mixture which could not be separated by TLC. Crystallization of the solid from ethanol gave motiol, mp 222–224°, $[\alpha]_D^{21}$ −39.6° [3].

Concentration of the mother solution yielded the second triterpene alcohol (**1**), mp 251–253°, $[\alpha]_D^{21}$ +157.8°, M^+ at *m/z* 426 ($C_{30}H_{50}O$), IRν_{max} cm^{-1}: 3610, 1080 (OH) and 1638, 892 (terminal methylene). The ^{1}H NMR spectrum of **1** showed six tertiary methyls at δ 0.72–0.98, one isopropenyl at 1.75 (3H, *s*) and 4.78 (2H, *br s*) and one C-3 proton at 3.19 (1H, *q*).

The mass spectrum of **1** exhibited intense peaks due to the cleavage of ring C at *m/z* 207 and 189 along with peaks at *m/z* 383 and 370, indicative of the hopane or moretane skeleton [4–6]. Moreover, in contrast with the ^{1}H NMR spectrum of moretenol isolated by the method described in the literature [7], the C-28 methyl signal of **1** appeared at δ 0.73, which was 0.04 ppm lower than the corresponding signal of the former compound [6, 8–10]. This strongly suggested the structure of **1** to be hopenol-b [hop-22(29)-en-3β-ol].

Acetylation of **1** furnished an acetate (**2**), mp 233–235°, $C_{32}H_{52}O_2$ (M^+ at *m/z* 468). The ^{1}H NMR signal of the C-3 proton quartet in **1** shifted to δ 4.52 in the spectrum of **2**, indicating an equatorial hydroxyl group in **1**. Oxidation of **1** gave a ketone (**3**), mp 220–222°, $C_{30}H_{48}O$ (M^+ at *m/z* 424), identical with hopenone-b which is known in nature [11].

Finally, the structure of **1** was proved by direct comparison with authentic hopenol-b [11, 12].

Compound **1** does not appear to have been previously isolated in nature, though it was derived from three natural triterpenes [11, 13, 14] and was recently biosynthesized by incubating a cell free system from *Acetobacter rancens*, *A. pasteurianum* or *Methylococcus capsulatus* with 2,3-epoxy-2, 3-dihydrosqualene [15–17].