Phytochemistry, Vol. 22, No. 2, pp. 602–603, 1983.
Printed in Great Britain.

0031-9422/83/020602-02$03.00/0

EFFECT OF COMPACTIN ON THE INCORPORATION OF MEVALONOLACTONE INTO GIBBERELLIC ACID BY *GIBBERELLA FUJIKUROI*

P. LEWER and J. MACMILLAN

Department of Organic Chemistry, The University, Bristol, BS8 1TS, U.K.

(*Received* 22 *June* 1982)

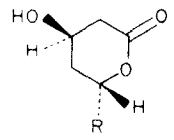
Key Word Index—*Gibberella fujikuroi*; compactin; gibberellin A_3; biosynthesis; inhibition.

Abstract—In *Gibberella fujikuroi*, strain GF-1a, the effect of the sodium salt of compactin on the incorporation of both radiolabelled acetate and mevalonate into gibberellic acid has been investigated. In each case, a concentration of 40 mg/l. caused a significant reduction in the incorporation.

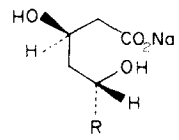
INTRODUCTION

The fungal metabolite compactin (**1a**) has been isolated from strains of *Penicillium brevicompactum*. It has been shown to be a highly potent, competitive inhibitor of hydroxymethylglutaryl coenzyme-A reductase and a cholesterol-lowering agent in several animals [1]. Thus, formation of cholesterol from acetate is inhibited by compactin in these systems, whereas that from mevalonate is unchanged.

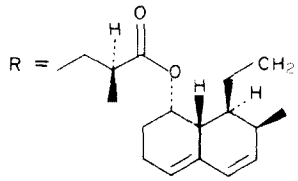
In the course of studying the metabolism of ^{13}C-labelled mevalonate in strains of *Gibberella fujikuroi* we wished to maximize the specific incorporation of the precursor into the fermentation products, which include several gibberellins. Hence the effect of compactin on incorporation of mevalonate into gibberellins, particularly GA_3, was of interest to us.



1a



1b



RESULTS AND DISCUSSION

Table 1 shows that, as expected, the incorporation of [2-^{14}C]mevalonolactone into GA_3 was much higher than that of sodium [2-^{14}C]acetate. By comparison of the figures for percentage incorporation into GA_3 in the presence and absence of the sodium salt of compactin (**1b**) the following results are also apparent. Incorporation of acetate was inhibited by 64.8%. However, incorporation of mevalonate was also inhibited, by 38.5%. These two figures show that 57.2% of acetate fed was converted to mevalonate, in the presence of, compared with in the absence of, compound **1b**.

Table 1. Incorporations of sodium [2-^{14}C]acetate and (3*R*)-[2-^{14}C]mevalonolactone into GA_3 by *Gibberella fujikuroi* strain GF-1a, in the presence and absence of **1b**

Compounds fed	% incorporation	Average % incorporation
[2-^{14}C]Acetate only	0.66	0.71
	0.76	
	0.70	
[2-^{14}C]Acetate + **1b**	0.39	0.25
	0.08	
	0.27	
[2-^{14}C]Mevalonolactone only	12.70	12.35
	12.35	
	12.00	
[2-^{14}C]Mevalonolactone + **1b**	7.20	7.60
	7.60	
	8.00	

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.

Acknowledgements—P.L. was supported by a S.E.R.C. studentship. We wish to thank Dr. I. K. Hatton, Beecham Pharmaceuticals Research Division, for a sample of compactin and Professor B. O. Phinney for the fungal culture.

REFERENCES

1. Endo, A. *J. Antibiotics* (1980) XXXIII, 33.
2. Geissman, T. A., Verbiscar, A. J., Phinney, B. O. and Cragg, G. *Phytochemistry* (1966) **5**, 933.

Phytochemistry, Vol. 22, No. 2, pp. 603–605, 1983.
Printed in Great Britain.

0031-9422/83/020603-03$03.00/0

TRITERPENOIDS FROM *HYPTIS SUAVEOLENS* ROOTS

TRIGUNA N. MISRA, RAM S. SINGH and JANARDAN UPADHYAY

Natural Products Research Laboratory, Department of Chemistry, Gorakhpur University, Gorakhpur, 273001, India

(*Received* 14 *May* 1982)

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