Biochemical Pharmacology, Vol. 25, 2217-2219. Pergamon Press, 1976. Printed in Great Britain.

PRELIMINARY COMMUNICATIONS

IDENTIFICATION OF THE GLUCURONIDES OF CANNABIDIOL AND HYDROXYCANNABIDIOLS IN MOUSE LIVER

D. J. Harvey, B. R. Martin and W. D. M. Paton

University Department of Pharmacology, South Parks Road, Oxford OX1 3QT, U. K.

(Received 11 June 1976; accepted 23 June 1976)

There has been much speculation in recent years on the nature of the conjugates formed during metabolic transformation of the cannabinoids (1) but to date, only one such compound, a diacetate, appears to have been reported (2). Indirect evidence obtained by hydrolysis of metabolic fractions with β-glucuronidase has suggested the presence of small amounts of glucuronide or sulphate conjugates formed from Δ^1- and Δ^6-tetrahydrocannabinol (THC) (3, 4) and cannabidiol (CBD) (5). Direct evidence for the formation of glucuronide conjugates by a number of other drugs has recently been obtained by examination of the intact conjugate with combined gas chromatography-mass spectrometry (GC-MS) (6-9). Using this method during the study of the metabolism of CBD, a cannabinoid reported to compete with Δ^1-THC for the drug metabolizing enzymes (10), we have characterized the glucuronide metabolites of CBD and several hydroxy-CBD's as the major *in vivo* hepatic metabolites in the mouse.

Male mice (Charles River, CD1, 23 g) were treated with CBD (100 mg/kg i.p.) suspended in Tween-80 and saline at 26 h and 2 h before sacrifice. The livers were removed, and the metabolites were extracted with ethyl acetate and partially purified by chromatography on Sephadex LH-20 in chloroform as described previously (11, 12). The derivatized metabolite fraction was then examined by GC-MS using a V. G. Micromass 12 B mass spectrometer interfaced to a Varian 2400 gas chromatograph (3% SE-30, 2 m x 2 mm) and a V. G. Data System type 2040. Gas chromatograms were run under temperature programmed conditions (170° to 280° at 2°/min) and mass spectra were recorded continuously from 190° at 3 sec/decade with a 2 sec inter-scan delay.

The GLC profile of the metabolic fraction as trimethylsilyl (TMS) derivatives contained a number of unconjugated metabolites (retention time 14-32 min), among which were the previously reported (13, 14) 6α- and 7-hydroxy-CBD. A second group of peaks which eluted from 38 to 50 min was produced by conjugates. The mass spectrum of the most abundant conjugate (retention time 39.5 min) exhibited a base peak at *m/e* 375 and contained ions at *m/e* 204 (5%), 217 (25%), 464 (7%) and 465 (5%) indicative of the

presence of glucuronic acid (7, 9). In addition the mass increases of 27, 18, 18, 36 and 36 atomic mass units (a. m. u.) respectively shown by these ions in the spectrum of the $\underline{d}_9$-TMS derivative (15) were also consistent with their possessing the structures of glucuronide fragment ions. The other major ions at $\underline{m/e}$ 458 (34%), 390 (38%), 386 (23%), 385 (14%), 337 (28%), 303 (23%) were produced from the aglycone and are characteristic of CBD. They also showed the appropriate shifts in the $\underline{d}_9$-TMS spectrum. The ion at $\underline{m/e}$ 458 (equivalent to the molecular ion of CBD), arose by transfer of a TMS group from the glucuronide moiety to the aglycone, a rearrangement reported to be specific for aromatic glucuronides (7, 9). The abundant ion at $\underline{m/e}$ 390 resulted from a retro-Diels-Alder elimination of C_5H_8 (68 a. m. u.) from $\underline{m/e}$ 458 (16), and the ions at $\underline{m/e}$ 386 and 385 were produced by cleavage of the CBD moiety with and without hydrogen rearrangement respectively.

Confirmation of the presence of the glucuronide carboxylic acid group was obtained by the preparation of the methyl ester-TMS derivative. This reduced the retention time to 39 min and produced a spectrum in which the glucuronide ions at $\underline{m/e}$ 375, 464 and 465 (TMS derivatives) were reduced by 58 a. m. u. showing replacement of a TMS group by a methyl group. Reduction of the conjugate with $LiAlD_4$ to a primary alcohol introduced two deuterium atoms (base peak of TMS derivative $\underline{m/e}$ 363), also indicating the presence of one acid group. Finally, hydrolysis of the metabolite fraction with β-glucuronidase at pH 5 in the presence of phosphate buffer (to inhibit sulphatase activity (17)) resulted in the disappearance of the conjugate peaks from the chromatogram and the appearance of unconjugated CBD (retention time 12.2 min).

In addition, the increase after enzymatic hydrolysis in the size of the GLC peaks produced by 6α- and 7-hydroxy-CBD suggested that these two metabolites could also conjugate with glucuronic acid. Peaks in the chromatogram of the TMS derivative of the unhydrolysed metabolite fraction at 42 - 45 min were subsequently identified as hydroxy-CBD glucuronides by the presence in their spectra of the glucuronic acid ions at $\underline{m/e}$ 204, 217, 375, 464 and 465 and the hydroxy-CBD ions at $\underline{m/e}$ 473, 474, 478 and 546. These glucuronides also formed methyl esters (base peak $\underline{m/e}$ 317) and were reduced to primary alcohols with $LiAlD_4$ with the incorporation of two deuterium atoms. In the spectrum of the largest hydroxy-CBD peak the presence of $\underline{m/e}$ 443 (loss of CH_2OTMS from the aglycone) together with the substituted tropylium ion at $\underline{m/e}$ 337 indicated that the conjugate was the glucuronide of 7-hydroxy-CBD. In the other hydroxy-CBD glucuronide spectra, ions characteristic of the position of hydroxyl substitution were not abundant enough to enable definite structural assignments to be made. In the spectra of all these compounds, the presence of ions at $\underline{m/e}$ 546 and 478, produced by transfer of a

TMS group to the hydroxy-CBD moiety, indicated that the glucuronic acid was conjugated to an aromatic rather than to an aliphatic hydroxyl group (7, 9).

In summary, the major *in vivo* metabolites of CBD have been identified by GC-MS as the glucuronides of CBD and 7-hydroxy-CBD. Indirect evidence provided by enzymatic hydrolysis also indicated the presence of the corresponding conjugate of 6α-hydroxy-CBD.

We thank the Medical Research Council for a Programme Research Grant, and the Wellcome Trust for personal support for B. R. M.

References

1. R. Mechoulam, N. McCallum, S. Levy and N. Lander. In Marihuana, Chemistry, Biochemistry and Cellular Effects (ed. G. G. Nahas), p. 3. Springer-Verlag, New York (1976).
2. F. Mikes, A. Hofmann and P. G. Waser, Biochem. Pharmac. 20, 2469 (1971).
3. L. Lemberger, S. D. Silberstein, J. Axelrod and I. J. Kopin, Science 170, 1320 (1970).
4. R. Mechoulam, Z. Ben-Zvi, S. Agurell, I. M. Nilsson, J. L. G. Nilsson, H. Edery and Y. Grunfeld, Experientia 29, 1193 (1973).
5. J. Christiansen and O. J. Rafaelsen, Psychopharmakologia 15, 60 (1969).
6. T. Imanari and Z. Tamura, Chem. Pharm. Bull, Tokyo 15, 1677 (1967).
7. S. Billets, P. S. Lietman and C. Fenselau, J. med. Chem. 16, 30 (1973).
8. M. G. Horning, C. Stratton, J. Nowlin, D. J. Harvey and R. M. Hill, Drug Metab. Dispos. 1, 569 (1973).
9. J. E. Mrochek and W. T. Rainey Jr., Anal. Biochem. 57, 173 (1974).
10. M. Fernandes, N. Warning, W. Christ and R. Hill, Biochem. Pharmac. 22, 2981 (1973).
11. D. J. Harvey and W. D. M. Paton. In Marihuana, Chemistry, Biochemistry and Cellular Effects (ed. G. G. Nahas), p. 93. Springer-Verlag, New York (1976).
12. D. J. Harvey and W. D. M. Paton, Res. Commun. Chem. Path. Pharmac., in press.
13. I. M. Nilsson, S. Agurell, J. L. G. Nilsson, M. Widman and K. Leander, J. Pharm. Pharmac. 25, 486 (1973).
14. B. Martin, M. Nordqvist, S. Agurell, J.-E. Lindgren, K. Leander and M. Binder, J. Pharm. Pharmac. 28, 275 (1976).
15. J. A. McCloskey, R. N. Stillwell and A. M. Lawson, Anal. Chem. 40, 233 (1968).
16. H. Budzikiewicz, R. T. Alpin, D. A. Lightner, C. Djerassi, R. Mechoulam and Y. Gaoni, Tetrahedron 21, 1881 (1965).
17. K. S. Dodgson and B. Spencer, Biochem. J. 55, 315 (1953).