the PMR spectrum of the di-*O*-trimethylsilyl derivative of **1** in CCl_4–C_6D_6 a benzene-induced shift of 0.5 ppm takes place for only one methoxyl group which implies [8, 9], that **1** has a methoxyl group *ortho* to an aromatic ring proton.

The ^{13}C NMR spectrum signals of **1** in DMSO-d_6 occur at similar resonances as those recently reported for chalcones [10, 11]: δ 193.4 (C=O); δ 160.0, 159.2, 158.5 1 C—Me, 2 C—OH); δ 143.1 (C-β); δ 136.2 (C-1); δ 131.2 (C-4); δ 130.2 (C-2, C-6, C-3′); δ 129.6 (C-3, C-5); δ 128.7 C-α); δ 106.6 (C-1′); δ 92.8 (C-5′); δ 61.1, 57.2 (2 OCH_3). In the ^{13}C-^1H coupled spectrum the C-5′ signal is a doublet of 162.6 Hz and is unaffected when the spectrum is recorded after D_2O exchange. This implies that C-5′ experiences no long range coupling with OH-2′ and is therefore *para* to C-2′ [12]. All other A ring carbons show a coupling of 2–5 Hz due to OH-2′ hydrogen bonded proton [12]. A *meta* coupling of 5 Hz between C-1′ and H-5′ is also present.

Fragmentation of **1** in the mass spectrometer follows fragmentation patterns similar to those described for chalcones [13]. Significant peaks appear at *m/e* 223 (M-77), 197 (M-103), 196 (M-104), 131, 104. 103, and 77. The rest of the spectrum is dominated by peaks at *m/e* 181, 167, 153, 139, 84, 69, and 49, all of which result from cleavage of ring A.

Acknowledgements—We thank Dr. J. O. Kokwaro of the University of Nairobi for identifying the plant and to Professor A. S. Perlin and Dr. N. Cyr of McGill University, Montreal for the ^{13}C-NMR spectra.

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Phytochemistry, 1978, Vol 17, pp. 824–826 Pergamon Press Printed in England

STRUCTURE OF TWO *C*-DIGLYCOSYLFLAVONES FROM *CANNABIS SATIVA**

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(*Revised received* 23 *November* 1977)

Key Word Index—*Cannabis sativa*; Cannabinaceae; marijuana; 2″-*O*-glucopyranosylorientin; 2″-*O*-glucopyranosylvitexin; orientin.

From a biologically active [11] aqueous extract of the aerial parts of *C. sativa* after pre-extraction with petroleum and MeOH, a flavonoid-rich butanol fraction has been obtained. Subsequent fractionation and purification by Sephadex and polyamide chromatography resulted in the isolation of three flavone *C*-glycosides A, B and C. Glycoside A with the lowest R_f (0.25) on cellulose TLC with *t*-BuOH–HOAc–H_2O(3:1:1) and the mp 266–267° was identified as orientin by comparison of the physical data with those of an authentic sample. The MS of the permethylated product (M^+ 564) was in accordance with the reported values [2]. The second flavonoid (glycoside B) had mp 218–230° and R_f 0.72. The third flavonoid (glycoside C) differed only slightly from the latter (mp 213.5–214) but was well separated by TLC (R_f 0.61). Compounds B and C could each be hydrolyzed by HCl compound B to a mixture of vitexin/isovitexin and compound C to orientin/isoorientin. In each case the 8-*C*-glucoside was a second product of hydrolysis, it was assumed that the compounds B and C were *O*-glucosides of vitexin and orientin respectively. Similar findings but without the presentation of any detailed physical and

*Part 8 in the series '*Cannabis sativa* L. (marijuana)'. For Part 7 see Segelman, A. B. and Segelman, F. P. (1976) *J. Chromatogr.* **123**, 9. Part 15 in the series flavone-*C*-glycosides. Presented at the 17th Annual Meeting of the American Society of Pharmacognosy, Cable, Wisconsin, 14 July 1976.

chemical data have been reported in a chromatographic study [3]. This was confirmed by the finding of seven alcoholic OAc-groups and 14 sugar protons in the NMR spectra of glycosides B and C-peracetates. The orientin and vitexin structures (8-*C*-hexosyl substitution) could also be deduced from the mass spectra of the permethylated glycosides, in which relatively weak peaks appeared at *m/e* 499 and 529 respectively [2]. UV shifts produced by NaOAc, NaOMe, $AlCl_3$ and H_3BO_3 solutions in MeOH showed that all phenolic OH groups were free [4]. The MS of the permethylated glycosides with peaks at *m/e* 311, 325 and 341 (glycoside B) and *m/e* 341, 355 and 371 (glycoside C) were in agreement with a trimethoxyflavone- and tetramethoxyflavone moiety [5]. This indicated clearly that the *O*-bound glucose was attached to one OH-group of the *C*-glucosyl moiety in compounds B and C, thereby forming flavone-*C*-biosides or *C*-diholosylflavones. Since signals for the 2″-OAc-groups in the region of $\delta = 1.70$–1.73 ppm were missing in the NMR spectra of both glycoside peracetates ($CDCl_3$) [6, 7], the 2″-OH-groups should also be the bonding position of the second sugar. Therefore, the structures for the *O*-glycosides B and C must correspond to 2″-*O*-glucopyranosylvitexin and 2″-*O*-glucopyranosylorientin respectively. Both compounds seem to be new, although the 2″-*O*-glucosides of isovitexin and isoorientin [8] and 2″-*O*-rhamnosides and *O*-xylosides of vitexin, isovitexin, orientin and isoorientin are known [6, 7, 9]. After completion of our work, a publication of the French group [10] on the flavonoids of *Cannabis* came to our knowledge, in which the authors, besides some other flavone glycosides, described two *O*-glucosides of orientin and vitexin respectively. From UV measurements they deduced that the glucose was attached to the C_7-OH group but no confirmatory evidence from MS or NMR was provided for a 7-*O*-glycosyl linkage. We have no evidence that such compounds other than the 2″-*O*-glucosides described above occur in *Cannabis* leaf.

EXPERIMENTAL

Materials and methods. Cannabis sativa L. plant material consisting of the air-dried flowering tops (leaves and twigs) and designated as Campus Mexican Female (CMF-261) was grown for 14 weeks and harvested at the University of Mississippi during the 1971 season. TLC and PLC: cellulose pre-coated plastic sheets with fluorescent indicator (Eastman) using: System 1: *t*-BuOH–HOAc–H_2O (3:1:1); System 2: *n*-BuOH–HOAc–H_2O (4:1:2); System 3: *n*-BuOH–HOAc–H_2O (4:1:5); System 4: EtOAc–HCOOH–H_2O (10:2:3); System 5; HOAc (15%) or on polyamide pre-coated plastic sheets (Macherey–Nagel) with fluorescent indicator using System 6; $CHCl_3$–MeOH–MEK (2:2:1). Charcoal (Norit 211) was purified as described previously [11]. NMR: Varian A-60 A. TMS as int. stand. in $CDCl_3$. EI-MS: MS-30 AEI; 70 eV; 2KV accelerating voltage; 300 μA total emmission; 180° ionization chamber temp.; 10^{-6}t vacuum.

Extraction and preparation of the flavonoid fraction. The ground twigs and leaves (18.8 kg) were successively extracted by percolation with petrol (bp 30–60°) (extract A), MeOH (extract B) and H_2O (extract C). The concd extract B (1.8 kg) was stirred for 12 hr with H_2O (18 l.) and filtered. This filtrate and extract C were each separately extracted continuously for 48 hr using *n*-BuOH. Separation and evapn of the BuOH phases gave flavonoid-rich residues that were combined on the basis of TLC to afford extract D. Extract D (400 g) in MeOH (4.5 l.) was stirred for 12 hr with purified charcoal (4 kg) and filtered. The charcoal filter cake containing the adsorbed flavonoids was successively washed with boiling MeOH (40 l.) and boiling H_2O (40 l.) to remove non-flavonoid impurities. The MeOH–H_2O washed charcoal was treated for 30 min with boiling 7% phenol in H_2O (40 l), filtered and the filtrate lyophilized to yield semi-purified extract E (40 g).

Chromatography and isolation of flavonoids. 30 g of extract E was chromatographed in 5 g portions (i.e. 6 column operations) on a column (5.5 × 50 cm) of Sephadex LH-20 (350 g) packed in MeOH-1,2-dichloroethane (7:3). Elution was performed with the same solvent mixture at a flow rate of 35 ml/hr. From each column a total of 450 5 ml fractions were collected, analyzed by by TLC, combined and evapd to afford 6 groups as follows: group 1 (fractions 1–80, 0.3 g); group 2 (fractions 81–140, 2.4 g); group 3 (fractions 141–180, 6.3 g); group 4 (fractions 181–250, 17.2 g); group 5 (fractions 251–295, 1.0 g); group 6 (fractions 296–450, 1.5 g). The residue from group 5 crystallized from 40% aq. MeOH. From MeOH yellow needles (268 mg) of orientin (glycoside A). Rechromatography of a portion (4.0 g) of group 4 residue on a column (5.5 × 50 cm) of Sephadex LH-20 (350 g) packed in MeOH and elution with MeOH at a flow rate of 35 ml/hr afforded a total of 320 5 ml fractions which were analyzed by TLC, combined and evapd to afford the following sub-groups: sub-group 4a (fractions 1–100, 0.03 g); sub-group 4b (fractions 101–164, 0.45 g); sub-group 4c (fractions 165–176, 0.15 g); sub-group 4d (fractions 177–246, 3.45 g); sub-group 4e (fractions 247–320, 0.02 g). A yellow solid (168 mg) was obtained during concn of the sub-group 4d fractions. Separation of this solid by PLC on 153 polyamide sheets (20 × 20 cm), elution with system 6 and crystallization (MeOH) of the two major bands afforded glycoside B (R_f 0.59) as an amorphous yellow solid (59 mg) and glycoside C (R_f 0.41) as pale yellow needles (63 mg).

Hydrolysis. 1–2 mg of the glycosides were hydrolyzed with 2 N HCl for 1 hr. After neutralization with Ag carbonate the soln was investigated by TLC on cellulose: system *i*-PrOH–Py–H_2O (6:2:2) and anilinephthalate as spray reagent: glucose $R_f = 0.57$; system 3 and UV det. for vitexin: $R_f = 0.45$, for orientin: $R_f = 0.27$.

Orientin. Glycoside A, yellow crystals, mp 266–276. (d) TLC cellulose (system, R_f) (1, 0.25), (2, 0.27), (3, 0.27), (4, 0.12), (5, 0.08) Polyamide-(6, 0.36). *Permethylated Orientin* MS *m/e* M^+ 560 (64% rel. int.), 546(7), 531(4), 385(100) 371(26), 355(11), 341(9) in accordance with published data [2]. Identical to authentic orientin by mmp, IR, UV and co-TLC.

Glycoside B. Yellow amorphous solid, mp 218–230° (d). TLC: cellulose-(system, R_f) (1, 0.72), (2, 0.69), (3, 0.63), (4, 0.61), (5, 0.75) polyamide-(6, 0.59). UV: λ_{max} 335 nm ε 15 469, 305 (*sh*) nm ε 12 578, 273 nm ε 16 016 nm (MeOH); 398. 332, 283 nm (NaOEt); 386, 347, 303, 277 ($AlCl_3$); 385, 340, 300, 277 ($AlCl_3$–HCl); 375, 305, (NaOAc); 333, 305 (*sh*), 268 (H_3BO_3 + NaOAc). IR (KBr): cm^{-1} CH—Ar: 2930; C=O (conj.): 1657;

Permethylated glycoside B. Prepared according to ref, [12] The PM product was washed with Et_2O, evapd to dryness and directly used for MS. *m/e* M^+ 734 (11% rel. int.), 545 (27), 544 (29), 529 (19), 516 (22), 515 (52), 501 (47), 499 (15), 485 (20), 355 (27), 241 (100), 327 (76), 325 (62), 311 (94), 298 (39), 219 (23), 187 (17), 155 (29).

Glycoside B-peracetate. Prepared according to ref. [13]. The PA-product was washed free from acids, dried and directly used for NMR. δ = 2.40–2.35 ppm (9 pr) OAc-5,7,4′; δ = 2.10–1.88 (21 pr) OAc3″,4″,6″2‴,3‴,4‴,6‴; δ = 5.50–3.1 (14 pr) CH-sugar; δ = 6.60–8.15 (6 pr) CH-aromatic.

Glycoside C. Pale yellow crystals, mp 213.5–214° (d). TLC: cellulose-(system, R_f) (1, 0.61), (2, 0.63), (3, 0.54), (4, 0.45), (5, 0.61) polyamide-(6, 0.41). UV λ_{max} 345 nm ε = 7880, 290 (*sh*) nm ε = 4348, 267 nm ε = 7337, 253 nm ε = 7391 (MeOH); 412, 317 (*sh*), 273 nm (NaOEt); 428, 333, 305 (*sh*), 277 nm ($AlCl_3$); 390, 357, 298 (*sh*) 277, 262 nm ($AlCl_3$–HCl); 270 (*sh*), 275, 324, 377 nm (NaOAc); 267, 305 (*sh*), 372 nm (H_3BO_3 + NaOAc); IR (KBr): cm^{-1} CH—Ar: 2940; conj. C=O: 1660; *Permethylated glycoside C m/e* M^+ 746 (4% rel. int.), 589 (7), 574 (17), 559 (27) 546 (13), 545 (43), 543 (17), 513 (15), 529 (12),

515 (10), 497 (11), 385 (50), 371 (100), 369 (25), 357 (60), 356 (32), 355 (68), 342 (30), 341 (78), 328 (15), 219 (5), 187 (20), 155 (13).

Glycoside C peracetate δ = 2.40–2.35 ppm (12 pr) OAc-5,7,3′,4′; δ = 21.2–1.89 (21 pr) OAc-3″,4″,6″,2‴,3‴,4‴,6‴; δ = 5.5–3.00 (14 pr) CH-sugar; δ = 6.65–7.85 (5 pr) CH-aromatic

Acknowledgements—This work was supported by US Public Health Service Grant DA-00328-03. We are grateful to Dr. Monique C. Braude, Chief Preclinical Pharmacology Division of Research, National Institute on Drug Abuse, Washington, D.C. for arranging procurement of the marijuana plant material that was used in this work.

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Phytochemistry, 1978, Vol 17 pp. 826–827 Pergamon Press Printed in England

LES FLAVONOÏDES DES GRAINES DE *LENS CULINARIS*

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Key Word Index—*Lens culinaris*; Leguminosae; flavones; tricetin; luteolin; flavonols; kaempferol; 5-deoxykaempferol; proanthocyanins; anthocyanins.

Abstract—Polyphenols isolated from *Lens culinaris* seed coats include tricetin, luteolin, a diglycosyldelphinidin and two proanthocyanidins. The cotyledons contain mainly a kaempferol glycoside and 5-deoxykaempferol.

INTRODUCTION

Dans le cadre d'une étude sur les rapports sol-plante, nous avons été amenés à analyser la composition flavonique des semences de la Lentille. Bien que l'objet de ces travaux soit physiologique, il nous a paru intéressant, devant l'indigence des données bibliographiques polyphénoliques [1] relatives à cette espèce, de rapporter ici les résultats de l'analyse structurale qui a révélé l'existence de 7 composés aglycones à l'intérieur de cet organe.

RESULTATS

Les téguments livrent une anthocyanine dont l'aglycone est facilement identifié après hydrolyse à la delphinidine. Le comportement chromatographique du produit naturel plaide en faveur d'une diglycosyldelphinidine dont la structure n'a pu être totalement élucidée du fait de la très faible teneur de ce composé (de l'ordre de 60 ppm). La chromatographie d'un extrait *n*-BuOH obtenu à partir d'un hydrolysat des téguments révèle l'existence de deux anthocyanes respectivement identifiées par comparaison avec des substances témoins à la delphinidine et la cyanidine. Ces deux composés proviennent de l'oxydation des proanthocyanes correspondantes, prodelphinidine et procyanidine présentes dans le rapport 60%, 40% pour une teneur globale de l'ordre de 1.5 p.mille. (Les cotylédons sont dépourvus de ces composés).

Téguments et cotylédons donnent à l'hydrolyse deux aglycones flavonols, l'un majeur identifié au kaempférol, l'autre mineur identifié au désoxy-5-kaempférol. La configuration structurale de ces deux composés a été assurée par comparaison chromatographique avec des témoins et par SM, et pour le kaempférol par spectrométrie UV en présence de réactifs. Le kaempférol est présent à l'état naturel sous forme d'hétérosides et principalement d'un tétraglycoside (en 3 et 7) dont la structure est actuellement à l'étude. La teneur de ce dernier atteint 150 ppm dans les cotylédons et 750 ppm dans les téguments.

Deux flavones majeures coexistent dans les téguments d'où elles ont pu être extraites sous forme d'aglycones libres. Il s'agit d'une flavone largement distribuée la lutéoline dont l'identification a été réalisée par confrontation chromatographique et spectrophotométrique avec un échantillon témoin, et d'une flavone très rare la tricétine dont c'est ici la quatrième mention à l'état naturel après celles de Lamer *et al.* [2] dans les feuilles de de *Thuja* et de *Lathyrus*, et de Beckmann et Geiger [3] dans les feuilles de *Metasequoia*: il semble en outre qu'il s'agisse là de la première citation de tricétine non liée.

Dans les téguments tricétine et lutéoline atteignent des