Résumé. Chez les lapins gris et blancs de la Nouvelle-Zélande, la concentration d'une enzyme semblable à la rénine est 16 fois plus grande dans l'utérus gravide presque à terme que dans l'utérus non-gravide et 9.5 fois plus grande que dans l'utérus après néphrectomie bilatérale. En tenant compte du fort accroissement de l'utérus gravide, le contenu total de l'enzyme en question est 114 fois supérieur à celui de l'utérus non-gravide et 24 fois plus grand que celui du rein.

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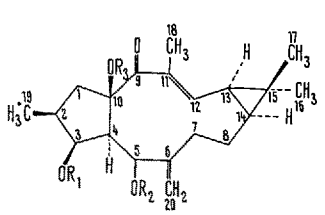
Further New Diterpene Esters from the Irritant and Cocarcinogenic Seed Oil and Latex of the Caper Spurge (*Euphorbia lathyris* L.)

From the hydrophilic neutral fraction of the seed oil of the caper spurge (*Euphorbia lathyris* L.) by multistage Craig distribution, the crystalline esters L_1, L_2 and L_3 and the resinous esters L_4 and L_5[1,2] have been isolated. L_1 and L_2 were identified as esters of the new macrocyclic diterpenes 6,20-epoxy-[3,4] and 7-hydroxy-lathyrol[5], respectively, and L_4 and L_5 as esters of the new tetracyclic diterpene ingenol[6,7]. Recently the structure of L_3 was clarified and further resinous (L_6, L_7) and crystalline (L_8) diterpene esters were isolated from the irritant and cocarcinogenic seed oil of the caper spurge. Also, a comparative investigation of the irritant latex of this species was undertaken.

Ester L_3, $C_{31}H_{38}O_7$ (MS), m.p. 156–158 °C is the diacetate-benzoate I of a new diterpene alcohol $C_{20}H_{30}O_4$[1,2]. I shows the following spectral data: UV (MeOH): λ_{max} = 229, 275 nm, ε = 16400, 15300; IR (KBr): 1730, 1705 (CO), 1640, 1613 (C = C-CO), 897 (C = CH_2), 705 cm^{-1} (C_6H_5). The NMR-spectrum ($CDCl_3$) indicates presence of two acetyl groups (δ = 2,23, 1,85; 2×3 H, S); the diamagnetic shift of the latter signal may be understood by the neighbourhood of the benzoyl group, the signal of which appears at' δ = 7,3–8,1 ppm (M). Further NMR data of I: H-12: 6,53, DD ($J_{12,13}$ = 11 cps, $J_{12,18}$ = 1–2 cps); H-5: 6,2, D ($J_{4,5}$ = 10 cps); H-3: 5,81, T ($J_{2,3} = J_{3,4}$ = 3,5 cps); H_2-20: 5,0, S, 4,78, S; H-1a: 3,6, DD ($J_{1a,1b}$ = 14 cps, $J_{1a,2}$ = 8,6 cps); H-4: 2,92, DD $J_{3,4}$ = 3,5 cps, $J_{4,5}$ = 10 cps); H-2: 2,3, M; H_3-18: 1,76, D ($J_{12,18}$ = 1–2 cps); H-13: 1,4, M; H_3-16, H_3-17: 1,22, S; H_3-19: 0,98 ppm, D ($J_{2,19}$ = 6,5 cps). The NMR data of the triester L_3 correspond to those of L_2, a tetraester of 7-hydroxy-lathyrol[5], with the exception that a signal of a geminal ester proton in position 7 is apparent. Thus the new diterpene alcohol is the parent of 6,20-epoxy- and 7-hydroxy-lathyrol, respectively, and therefore called lathyrol (II). Because of the close relationship of II with 7-hydroxy-lathyrol, *trans*-configuration of $\Delta^{8,9}$ and the absolute configuration as determined for the latter by X-ray diffraction analysis[5] may be adopted also for II. The positions of the three acyl groups in I remain to be determined. The saturated hydrocarbon corresponding to lathyrol (II) is proposed to be called lathyran.



I: R_1-R_3 = 2 $COCH_3$, 1 COC_6H_5
II: $R_1 = R_2 = R_3$ = H
III: $R_1 = R_2$ = $COCH_3$, R_3 = H
IV: R_1-R_3 = 2 $COCH_3$, 1 COC_5H_4N

By hydrolysis of I (0,5 m KOH in methanol) lathyrol (II), $C_{20}H_{30}O_4$ (MS), m.p. 168–169 °C is obtained. It is acetylated with Ac_2O/py to yield lathyrol-3,5-diacetate III, m.p. 134–136 °C, NMR ($CDCl_3$): H-3: 5,55, T; H-5: 5,87, D; OH-10: 2,9–3,4 ppm (broad).

L_6 (resinous, MS: parent ion *m/e* = 548) is a mono-ester of ingenol[7] with the highly unsaturated $\Delta^{2,4,6,8,10}$-penta-en-tetradecanoic acid. Transesterification of L_6 (1% $NaOCH_3$ in methanol) yields ingenol and the methyl ester $C_{13}H_{17}COOCH_3$ (MS) which, on hydrogenation with Pd/C, leads to the methyl ester of tetradecanoic acid identified by gas-liquid chromatography and mass spectrum.

Ester L_7 (resinous, MS: parent ion *m/e* = 580) was not further investigated because of lack of material. According to its UV-spectrum (MeOH) (λ_{max} = 278 nm, ε_{max} = 11600), a structural relationship of its parent alcohol to lathyrol (II) is indicated.

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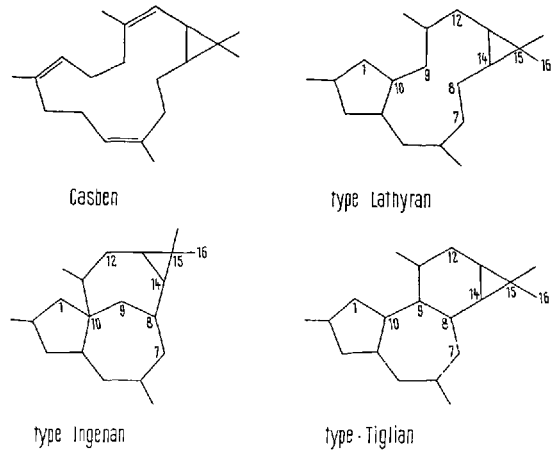
Ester L_8, m.p. 198–203 °C, contains nitrogen: $C_{30}H_{37}O_7N$ (MS) and is the diacetate-pyridine-3′-carboxylate IV of lathyrol. UV (MeOH): λ_{max} = 218,5 271,5 nm, ε = 14700, 15400. IR (KBr): 1740, 1715 (CO), 1647, 1623 (C = C–CO), 1590 (C = N), 902 (C = CH_2), 740, 700 cm^{-1} (C_5H_4N). With the exception of the signals of the aromatic protons, the NMR spectrum of IV ($CDCl_3$) is identical with that of I. Instead of the multiplet of the benzoyl group as in I, the NMR spectrum of IV shows the signals of the protons of nicotinic acid: H′-2: 9,25, D ($J_{2,4}$ = 1 cps); H′-6: 8,75, DD ($J_{5,6}$ = 2,5 cps, $J_{4,6}$ = 1 cps); H′-4: 8,25, M ($J_{4,5}$ = 4 cps, $J_{4,6}$ = 1 cps, $J_{2,4}$ = 1 cps); H′-5: 7,4 ppm, DD ($J_{4,5}$ = 4 cps, $J_{5,6}$ = 2,5 cps). The relative positions of the three ester groups in IV remain to be established.

In the acetone extract of latex of *E. lathyris* collected from plants in their second year, apparently none of the 8 diterpene esters L_1–L_8 isolated from the seed oil is present. Especially, no esters of lathyrol type diterpenes were found. However, a mixture of ingenol esters with highly unsaturated fatty acids (C_{10}: 2⊿ and 3⊿; C_{12}: 2⊿ and 3⊿, mass-spectrometrically) was isolated.

The esters of ingenol with a free hydroxyl group in 20-position exhibit considerable irritant (L_5, L_6 and mixture of esters from latex) and cocarcinogenic activities (L_5) in the mouse (see [1, 2, 6]). L_4 i.e. ingenol-20-hexadecanoate and also the esters of lathyrol and its derivatives (L_1, L_2, L_3, L_7, L_8) are inactive in the biological assays mentioned above (see [1, 2, 6]).

Recently an a further derivative of the macrocyclic parent hydrocarbon lathyran has been isolated from an Euphorbiacea: Bertyadionol[11] form a Bertya species. The structural relationship between the macrocyclic skeletons of casbene[10], lathyran, tiglian and ingenan[3–6], as visualized in the scheme above, may indicate the existence of hitherto unknown biosynthetic pathways of diterpenes in Euphobiaceae: for example from geranyl-geraniol-pyrophosphate they may form parent alcohols of the lathyran type (*E. lathyris*, Bertya sp.[11]), tiglian type (*Croton tiglium*[1], *E. triangularis*[8], *E. cooperi*[9]) and ingenan type (*E. lathyris*, *E. ingens*[7]).



Zusammenfassung. Ein Diacetat-Benzoat und ein Diacetat-Nicotinoat des neuen Diterpens «Lathyrol» sowie weitere Ester des Diterpens Ingenol wurden aus dem Samenöl bzw. dem Latex von *Euphorbia lathyris* L. isoliert. Die chemische Struktur von Lathyrol wurde mittels spektraler Daten aufgeklärt. Lathyrol ist die Muttersubstanz der beiden bereits früher aus *E. lathyris* isolierten makrozyklischen Diterpene 6.20-Epoxy-lathyrol und 7-Hydroxy-lathyrol. Es wird ein Biogeneseweg für Diterpene aus Euphorbiaceen vorgeschlagen.

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Circadian Variations of Muscle Metabolites

On the basis of several reports, it could be established that glycogen[1] and protein[2] metabolism in muscle has a very fast turnover and also that it is under hormonal control. Nevertheless, even when it is known that the levels of circulating hormones are far from being constant[3–6], there is a scarcity of available data concerning the spontaneous variation of muscle metabolites concentration during the 24 h period. This information might provide important clues for the choice of any experimental design schedule. Therefore, we considered it important to establish whether the content of substances such as DNA, total protein and glycogen in muscle tissue vary or remain unchanged during the 24 h period. The results obtained are presented in this paper.

Material and methods. Female mice of the C3H-S strain, 6 weeks old, from the Instituto de Embriología, Biología e Histología, Facultad de Ciencias Médicas, Universidad Nacional de La Plata, were used throughout the experiments. In their 3rd week of age they were caged in groups of 10 in a room at a temperature of 25 ± 1°C with water and food ad libitum and illumination (fluorescent light 40 W) from 06.00 to 18.00 h alternating with 12 h darkness. Mice have nocturnal habits, and feed during the dark period, as has been previously demonstrated[7]; thus, the dark (18.00–06.00) and light (06.00–18.00) periods are named activity and rest periods, respectively.

Lots of 7 animals each were killed by decapitation at 00.00, 04.00, 08.00, 12.00, 16.00 and 20.00 h on different days. The average body weight in each lot was carefully kept around 20 g. The diaphragms were quickly removed, blotted between filter paper and weighed. Homogenization of the tissue, either for protein or DNA determination, was done in 2 ml of isotonic saline. DNA was extracted

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