The compound **2** ($C_{27}H_{40}O_4$) showed the following spectral data: UV λ_{max} (CH_3OH) 244 nm ($\varepsilon = 11.800$); IR ν_{max} 3450, 1654, 1622 and 1604 cm^{-1}; MS m/z 410 (5%, $M^+ - H_2O$), 395 (11%), 353 (20%), 327 (12%), 253 (53%), 121 (100%); PMR: δ ($CDCl_3$), 7.07 (H-1, d, J = 10 Hz), 6.21 (H-2, dd, J = 10 and 2 Hz), 6.05 (H-4, bs), 5.68 (2H), 4.56 (H-16, w/2 = 18 Hz), 3.92 (2H, ABq J = 12 Hz), 1.43 (3H, 21-H's, s), 1.24 (3H, 19-H's, s), 0.88 (6H, d, J = 7 Hz, isopropyl Me's); CMR (table).

The above data were very similar to those of **1,** significant differences being observed in the MS (m/z 410, $M^+ - H_2O$), PMR (absence of the 3H doublet at δ0.96) and CMR (absence of the off-resonance quartet due to C-28 at δ17.7) spectra; these differences were easily explained by the absence of the methyl group at C-24.

The structure **3** has been suggested on the basis of the following evidence: UV λ_{max} (CH_3OH) 241 nm ($\varepsilon = 15.000$), IR ν_{max} 3450, 1660 and 1610 cm^{-1}; MS m/z 426 (4%, $M^+ - H_2O$), 411 (51%), 383 (12%), 355 (82%), 329 (32%), 255 (100%), 124 (14%), 123 (18%); PMR δ ($CDCl_3$) 5.74 (H-4, s), 5.65 (2H), 4.57 (H-16, w/2 = 18 Hz), 3.92 (2H, ABq, J = 12 Hz), 1.43 (3H, 21-H's, s), 1.22 (3H, 19-H's), 0.96 (3H, d, J = 7 Hz, 28-H's), 0.87 (3H, d, J = 7 Hz), 0.85 (3H, d, J = 7 Hz); CMR (table).

Comparison of these data with those for **1** allowed the conclusion that in **3** there was a different arrangement of the ring A, characterized by the presence of a Δ^4-3-ketone.

The same chromophore was found in the mixture of **4** and **5,** which were not separable by the usual chromatographic techniques. The MS data[12] were in accordance with a C-27 steroid (m/z 412, $M^+ - H_2O$) characterized by the same oxidation pattern as **3,** but the presence in the PMR spectrum[12] of an AB quartet at δ3.91 and of 5 signals attributable to methyls, suggested the possibility of a 1:1 mixture of 2 compounds. The CMR spectrum (table) confirmed this suggestion; it showed all the signals easily asignable, by comparison with ^{13}C resonances of **2** and **3,** to the carbon skeleton of **4** and, in addition, signals at δ136.1 (d), 132.7 (d), 38.1 (d), 29.8 (t), 20.3 (q) and 11.8 (q) due to the presence of a further component with the same carbon nucleus of **4** but with the side chain arranged as depicted in **5.**

The reported spectral data exclude for all the new compounds a possible C-19 hydroxylation. In fact in the CMR spectra (table) the resonances of the C-18 functionalized carbons are constant at δ60.1, while those of C-19 are strongly influenced by the arrangement of the ring A.

All these unusual C-18 hydroxy steroids are biogenetically related to guggulsterol III (**6**), which occurs at the same time in the same organism[7], and which was previously found in the biologically active extracts of the three *Commiphora mukul*[11].

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Reversible impairment of hepatobiliary function induced by streptozotocin in the rat[1]

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Summary. The effect of streptozotocin (SZ) on hepatobiliary function was studied in rats on the 1st, 7th and 15th days of treatment. Serum glucose increased significantly on the 1st day, and then remained high. Bile flow, bile acids output and BSP biliary excretion were significantly decreased on the 1st day of treatment, whereas serum sorbitol dehydrogenase was increased. All the parameters tested apart from serum glucose tended to normalize with time. The results suggested a transient toxic effect of SZ on the hepatocyte.

Streptozotocin (SZ) is a highly effective cytotoxic agent for pancreatic β-cells[3] probably due to a high capacity of these cells for accumulating the compound[4]. SZ administration in animals results in an irreversible diabetic state which develops by 24 h post-injection[5]. Besides being an effective diabetogenic substance, SZ possesses carcinogenic, antibacterial and antitumoral properties[4]. Despite the enormous amount of information concerning metabolic disorders in the liver related to SZ-induced diabetes very little is known regarding the hepatobiliary function in SZ-treated animals. It was reported that SZ-induced diabetes in rats showed a decrease in total pool of bile acids[6] as well as in bile flow and biliary excretion of drugs[7].

Furthermore, some modifications of the hepatocyte membrane[8] and a decrease in UDP-glucuronyltransferase activity related to an alteration in the membrane environment of

Parameters tested in control and SZ-treated rats

Parameter	Control rats (6)	SZ-treated rats 1st day (6)	7th day (5)	15th day (4)
Serum glucose (g/l)	1.21 ± 0.05	4.89 ± 0.55[a]	6.01 ± 1.24[a]	5.48 ± 1.52[a]
Bile flow (µl/min/g of liver)	2.38 ± 0.17	1.21 ± 0.08[a]	1.38 ± 0.10[a]	1.72 ± 0.32
Bile acid output (nmol/min/g of liver)	59.06 ± 5.96	39.68 ± 2.73[a]	62.42 ± 11.94	65.85 ± 6.21
Serum SDH, (U/l)	1.29 ± 0.22	7.17 ± 1.19[a]	2.83 ± 0.68[a]	1.88 ± 0.79
Body wt	243 ± 10	221 ± 11	231 ± 5	216 ± 9[a]
Liver wt	8.14 ± 0.39	7.67 ± 0.04	8.25 ± 0.18	8.98 ± 0.50
(Liver wt/body wt) 100	3.38 ± 0.19	3.52 ± 0.19	3.56 ± 0.06	4.17 ± 0.14[a]

Results are mean values ± SE. [a] Indicates a significant difference (refer to the statistical significance of the differences between treated and control groups). The number of animals is given in the parentheses. SDH: sorbitol dehydrogenase activity.

the enzyme[9] were described in SZ-induced diabetes. Therefore in this study several parameters of hepatobiliary function were investigated in rats at various times after SZ treatment.

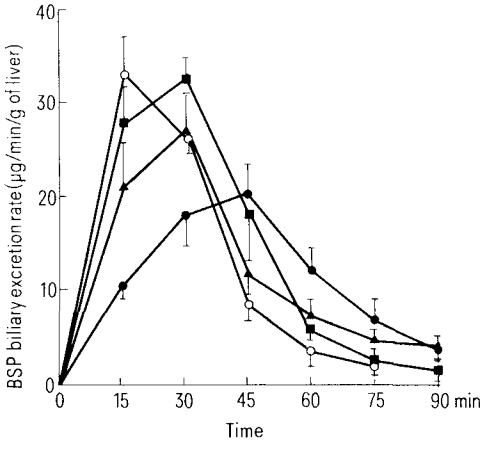
Materials and methods. Animals and treatment. Male Wistar rats (180–280 g) were used and randomly divided into 2 groups. Animals from one group received SZ (Sigma Chemical Co, USA) i.v. (50 mg/kg b.wt dissolved in 0.05 M citrate buffer, pH 4.5[10]). Rats from the other group were injected with buffer alone and used as controls. The rats were allowed free access to water and were fed ad libitum until being used (24 h, 7 days and 15 days after SZ injection, and 24 h after citrate buffer injection in the controls). On the day of the experiment animals were anesthetized with sodium pentobarbital (50 mg/kg b.wt, i.p.) and the bile duct and a femoral vein were cannulated with a PE-10 polyethylene catheter (Intramedic, USA).

Experimental procedure. The bile was collected every 15 min for 60 min, in darkness. To prevent hypothermic variations of bile flow[11], the rectal temperature was maintained at 38.0 ± 0.5 °C throughout the experiment with a thermostatically controlled heating lamp. Then a single dose of sulfobromophthalein (BSP, 60 mg/kg b.wt) (Sigma Chemical Co. USA) was injected i.v. and bile collection continued every 15 min for another 60 min. At the end of bile collection, blood was obtained by heart puncture, and the liver was removed and weighed. The ratio liver wt/body wt was calculated.

Assay techniques. The bile flow (BF) was estimated gravimetrically and expressed as µl/min/g of liver. Bile acids (BA) were assayed with 3-α-hydroxysteroid-dehydrogenase (Sigma Chemical Co. USA) according to Talalay's method[12] modified by Berthelot et al.[13]. The output of BA (nmol/min/g of liver) was calculated for each rat. BSP was measured in the bile samples by spectrophotometry at 580 nm after alkalization. The relative amounts of free and conjugated BSP in bile were estimated by thin-layer chromatography and spectrophotometry[14]. Serum glucose was measured enzymatically[15] (GOD-PAP test, Boehringer Mannheim, FRG). The activity of serum sorbitol dehydrogenase (SDH), was also determined[16,17] (Boehringer Mannheim, FRG). Student's t-test was used in the comparison of data. The level of significance was chosen as $p < 0.05$.

Results and discussion. The results are presented in the table. On the 1st day of SZ treatment serum glucose increased significantly. Hyperglycemia in 7-day and 15-day diabetic rats was about the same degree as on the 1st day. BF and BA output diminished significantly on the 1st day after injection of SZ whereas serum SDH was increased at this time. All the parameters tested excepting serum glucose showed the greatest variation on the 1st day of the injection of SZ. Then a clear tendency to return to the values seen in the controls was observed on the 7th and 15th days post injection. Thus no differences existed between control and treated rats either for BA output on the 7th day of treatment or for BF and serum SDH on the 15th day of SZ administration. Body wt diminished in diabetic rats but the decrease was not accompanied by loss of liver mass, resulting in an increase in the liver wt/body wt ratio relative to controls as described[18]. BSP biliary excretion was significantly delayed on the 1st day of SZ treatment but then a pattern similar to that of controls was observed on days 7 and 15 post injection (fig.). The proportion of conjugated BSP in bile did not show differences between controls (82.6 ± 1.0%) and treated animals (83.4 ± 1.0%).

The results suggest that SZ administration to rats induced a transient impairment of the processes involved in bile formation. Since BA are considered to be one of the solutes generating BF[19] we can assume that BA-dependent flow was impaired in SZ-treated rats. However, despite the fact that BA output was normalized on the 7th day of treatment, BF depression still continued, although it was less pronounced. Thus we can not discard the possibility that BA-independent flow[20] was also altered by SZ administration. In this connection the biliary excretion of BSP, which is greatly influenced by BF variations[21], may also be impaired by SZ as described in this paper. The sharp increase of serum SDH, a sensitive indicator of liver damage in rats[22], may be due to a toxic effect of SZ on the hepatocyte. Supporting this assumption it was observed



Sulfobromophthalein biliary excretion in control and SZ-treated rats. Results are mean ± SE for 6 control rats (▲-▲), 6 SZ-treated rats on the 1st day (●-●), 5 SZ-treated rats on the 7th day (■—■), and 4 SZ-treated rats on the 15th day (○-○) of treatment. BSP biliary excretion on the 1st day was significantly decreased during the early time points (15 and 30 min). There were no significant differences between groups in the total amounts of BSP excreted during 90 min (controls: 1400 ± 99.8 µg/g liver; SZ-treated rats: 1174 ± 93.4 µg/g liver on the 1st day, 1483 ± 197.8 µg/g liver on the 7th day, and 1429 ± 266 µg/g liver on the 15th day of treatment).

that drug metabolism in isolated hepatocytes from diabetic rats was inhibited at shorter durations of diabetes; such an effect was attributed to some transient effect of SZ[18]. It was reported that focal hepatic necrosis in rats could be observed 2-36 h after the injection of SZ, but no other evidence of gross direct toxic damage in liver[23].

In conclusion, as impaired hepatobiliary function was not correlated with hyperglycemia we can assume that it may be a consequence of a direct effect of SZ rather than of the induced diabetic state. Such an effect must be considered in studies on hepatic metabolic disorders in experimental diabetes caused by SZ.

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Oviposition rhythm of individual *Drosophila pseudoobscura*

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Summary. *D. pseudoobscura* oviposits mostly during the day with some oviposition at night. Oviposition varies from tight clustering to loose scattering from different individuals. Daily oviposition ranges from good to poor to no eggs over 24 h. Although this species was an excellent model for the eclosion rhythm it does not serve as a good model for continued study of the oviposition rhythm under present experimental conditions.

Eclosion rhythm studies of *Drosophila pseudoobscura*[2,3] are now part of the historical support for our understanding of the 'biological clock'. However, *D. melanogaster* is by far the more popular choice of the two species when using physiological and genetic analysis[4-6] of the way the clock works. Another rhythm, the oviposition rhythm, has been investigated in *D. melanogaster*[7,8] and it appears to be promising for study in greater depth. Because of its past role in clock studies it was thought prudent to investigate the oviposition rhythm of *D. pseudoobscura* and compare it to the oviposition rhythm of *D. melanogaster*. Oviposition information about *D. pseudoobscura* is sparse[9,10].

My report will show that the oviposition rhythm of individually-housed *D. pseudoobscura* is greatly different from that of *D. melanogaster*. I also report an improvement on the method previously used[8].

Methods. A stock culture of wild-type *D. pseudoobscura* was generously supplied by C.S. Pittendrigh. The same apparatus, lighting conditions, housing, and culture conditions previously described[8] were adapted for the present study. A conveyor belt moved a food tray 2.54 cm per h while individually housed flies descended from a fixed chamber to eat and oviposit in the food. The food was pressed into channels carved into a plastic tray.

During the study, an improved method was developed, as follows. Tightly woven dark blue polyester cloth was cut to fit the surface of the food tray. The cloth was moistened with a boiled solution consisting of 10 g soluble starch, 20 ml corn syrup (Karo, CPC International Inc. Englewood Cliffs, N.J.) in 1 l of H_2O. When stretched on the tray the moist cloth was painted with a yeast (Red Star, Milwaukee, WI) suspension (10 g/100 ml). On the conveyor belt, 25 individually housed flies convert the food tray into an egg tray. The process of making ovigraphs from the egg tray was the same as reported earlier[8]. The old method was used with *D. pseudoobscura* several times before the newer cloth method was adopted. Only results using the cloth method will be reported, along with 1 typical result.

Results. The cloth food tray method is an improvement over the channel method for feeding and oviposition. Eggs on the dark cloth are clearly visible without optics, thus giving an immediate assessment of an experimental run when trays are changed each day. Search and recording time is greatly reduced because eggs are no longer embedded in the food in the channels but are on the cloth surface. A dry egg cloth could be saved if necessary for a voucher specimen of an experiment, because dry eggs