composed of different fractions or that "degradation" occurred during the storage period of lot WPTY 175B. No matter which hypothesis is retained, the product has not lost its activity as far as emulsifying or surface-active properties are concerned.

Nevertheless, the average molecular weight of an emulsifier ought to be known in order to determine its surface excess and to produce the curve of the surface pressure *versus* "molecular" cross-sectional area of the emulsifier (7). Relying on the reported molecular weight can generate misleading values for the aforementioned parameters, especially if the data are obtained from different batches of the same emulsifier. No comparison can be made between the behavior of different lots of one product unless a correction is made for the difference in their molecular weights.

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Alloxan Analogues as Potential Pancreatic-Imaging Radiopharmaceuticals

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Received August 19, 1982, from the **Center for Health Sciences, Lehigh University, Bethlehem, PA 18015* and the ‡*Biochemistry Department, McNeil Laboratories, Spring House, PA 19477.* Accepted for publication January 10, 1983.

Abstract □ Three families of alloxan derivatives, 5-arylthiobarbituric, 5-aryliminobarbituric, and 5-aryldialuric acids, were prepared as prospective radioiodine-transporting radiopharmaceuticals for the delineation of pancreatic insulinomas. Members of each class were screened for effects on blood sugar levels in a rat glucose tolerance assay. Transient hyperglycemia was observed with 5-(2,4-dichlorophenyl)iminobarbituric acid. No agent evaluated induced permanent diabetes at the doses tested.

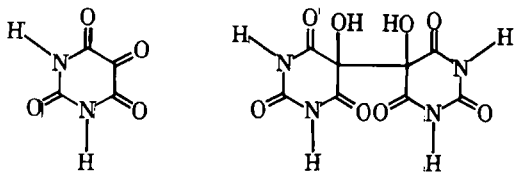
Keyphrases □ Alloxan—analogues, potential tumor-imaging agents, pancreatic insulinomas, effect on blood glucose levels, rats □ Tumor-imaging agents—alloxan analogues, pancreatic insulinomas, effect on blood glucose levels, rats

Alloxan and several of its derivatives are diabetogenic (1–3). While no definitive structure–activity studies have been reported, it appears that alloxan, alloxan monohydrate (II), alloxantin (I), and dialuric acid (III), without bulky substituents on nitrogen, are all able to induce frank diabetes (1–3). Some evidence supports a direct effect on the pancreas, for degranulation of β-cells is observed; [^{14}C]alloxan was shown to concentrate in the islet cells by autoradiography (4–7).

Development of radiopharmaceuticals for the scintigraphic imaging of occult pancreatic malignancies has been a goal of these laboratories for several years (8–11). In at least one previous study, it has been shown that substances with direct effects on serum glucose levels can, if radioisotopically labeled, preferentially delineate insulinomas (12, 13). Alloxan analogues would appear worthy of investigation in this regard.

Three classes of alloxan derivatives have been prepared which differ from the parent molecule by possessing an aryl moiety at C-5. This pendant aromatic ring would provide a suitable locus for the attachment of radioiodine in the chemical agent actually selected for the tumor-imaging study. As an initial screen to select analogues for isotopic labeling, a glucose tolerance test was performed to assay diabetogenic potential. This procedure has been described previously (14). Candidates were selected from the three classes synthesized: 5-arylthiobarbituric acids (IV), 5-aryliminobarbituric acids (V), and 5-aryldialuric acids (VI).

A new and more facile synthesis of 5-arylthiobarbituric acids (IVa–c) was developed from triphenylphosphine–alloxan adducts (VII), whose synthesis was described in an earlier publication (15), and aromatic thiols (Scheme



alloxan

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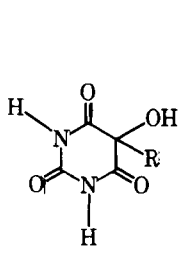
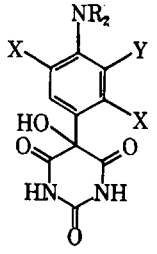


II: R = OH
III: R = H



VIa: R = X = Y = H
VIb: R = Me, X = Y = H
VIc: R = Y = H, X = OMe
VId: R = Y = H, X = OEt

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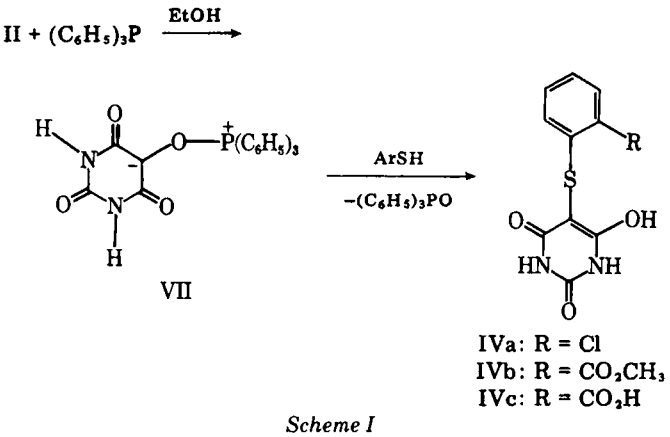


Scheme I

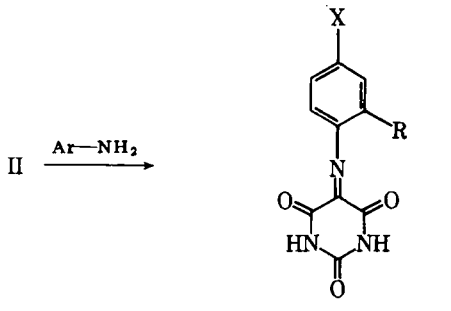
I). The mercapto function displaces the triphenylphosphine oxide to generate the arylthiobarbiturates. Since the alloxan–triphenylphosphine adduct is available in nearly quantitative yield, the overall conversion to the desired products (IVa–c) is more convenient than the published route, which requires the preparation and purification of 5-chlorobarbituric acid and subsequent nucleophilic displacement of the chloro group by an arylthiolate anion (16).

5-Aryliminobarbituric acids have been previously prepared from alloxan anhydride, which was obtained by dehydration of the alloxan monohydrate (II) (17). Here, the alloxan anhydride, generated *in situ* by treatment of the monohydrate with glacial acetic acid, was trapped by the aromatic amine (Scheme II).

The dialuric acids (VIa–g) were generated by condensation of anilines, possessing an unsubstituted *para*-position, with alloxan monohydrate in glacial acetic acid. Even primary aromatic amines with open *para*-positions condense preferentially at the *para* site (to form dialuric acids) rather than at the amino group (to form iminobarbiturates) (17–20).

EXPERIMENTAL[1]

5-Arylthiobarbituric Acids (IVa–c)—Equimolar amounts (5.0 mmol) of the triphenylphosphine–alloxan adduct (VII) (15) and the requisite aromatic thiol (see Scheme I) were refluxed for 2 h in 50 mL of 1:1 glacial acetic acid–absolute ethanol. The solvent was evaporated *in vacuo* to approximately one-half the original volume, the mixture was chilled, and the product was removed by filtration. The resulting thiobarbiturates were recrystallized by dissolution in a minimal quantity of boiling ethanol and reprecipitation by dropwise addition of 1:3 benzene–petroleum ether (60–110°C). The 5-arylthiobarbituric acids were characterized by broad—OH and —NH absorption in the IR between 2800 and 3300 cm^{-1} and by carbonyl absorption at 1725 ± 5, 1705 ± 5, and 1645 ± 5 cm^{-1}. Yields are reported in Table I.



Scheme II

5-Aryliminobarbituric Acids (Va–d)—Equimolar amounts (6.25 mmol) of alloxan monohydrate (II) and the requisite aniline (see Scheme II) were heated at reflux in 15 mL of glacial acetic acid for 0.5 h. The mixture was cooled (0°C); Vb–d crystallized as red-purple solids and Va separated as an oil (crystallized from dioxane–benzene). The solids, Vb–d, were washed with a minimum of warm benzene, filtered, and dried *in vacuo* to yield analytical specimens. The 5-aryliminobarbituric acids were characterized by composite —OH and —NH absorption between 2820 and 3380 cm^{-1} and by carbonyl and imine absorption at 1750 ± 5, 1720 ± 5, and 1680 ± 5 cm^{-1}. Yields are reported in Table I.

5-Aryldialuric Acids (VIa–d)—The dialuric acids were prepared by a previously described method (20) from equimolar amounts (6.25 mmol) of alloxan monohydrate and an aniline with an unsubstituted *para*-position in 20 mL of glacial acetic acid at 25°C. The mixture was stirred at ambient temperature for 30 min, chilled, and the product removed by filtration. The resulting crystalline material was washed with 2 × 10-mL portions of cold glacial acetic acid and then dried (0.01 mm). Yields are reported in Table I. The IR spectra displayed —OH and —NH absorption between 3100 and 3600 cm^{-1} and carbonyl absorption at 1730 ± 5 and 1710 ± 5 cm^{-1}.

[1] Analyses were performed by Dr. G. I. Robertson, Florham Park, N.J. Melting points were determined between glass disks on a Fisher-Johns Apparatus and are reported uncorrected. NMR spectra were obtained on a Perkin-Elmer Hitachi R20A spectrometer and are calibrated against tetramethylsilane. Infrared spectra were taken in 1–2% KBr disks on a Perkin-Elmer Model 257 infrared spectrometer.

Table I—Alloxan Analogues Investigated as Potential Pancreatic Radiopharmaceuticals

Compound	Formula[a]	mp, °C dec.	Yield, %
IVa	$C_{10}H_7ClN_2O_3S$	268.0–268.5	34
IVb	$C_{12}H_{10}N_2O_5S \cdot \frac{3}{4}H_2O$	235	17
IVc	$C_{11}H_8N_2O_5S \cdot \frac{1}{2}H_2O$	247–248	39
Va	$C_{10}H_5Cl_2N_3O_3$	251–253	40
Vb	$C_{10}H_6BrN_3O_3 \cdot \frac{1}{4}H_2O$	234	64
Vc	$C_{10}H_7N_3O_4 \cdot \frac{1}{4}H_2O$	>300	94
VIa		252–253[b]	67
VIb		232–233[c]	72
VIc	$C_{12}H_{13}N_3O_6$	267–270	83
VId	$C_{14}H_{17}N_3O_6$	225–226	85

[a] Elemental analyses for C, N, and H were within 0.4% of the theoretical values for all compounds. [b] Lit. mp 248°C (dec.) (ref. 19). [c] Lit. mp 230°C (dec.) (ref. 19).

RESULTS AND DISCUSSION

Six of the materials prepared in this study, including representatives of each of the three compound classes, underwent a rat glucose tolerance screen. The assay was performed as previously described (14) on sets of three male Sprague–Dawley (210–250 g) rats fasted for 48 h prior to intraperitoneal injection of the test compound as a suspension in 0.5% methylcellulose. Animals were administered glucose (1.0 g/kg of body weight) orally 30 min after injection of the test compound. Blood, withdrawn from the tail vein at the time of glucose administration and at 1, 2, 3, 4, 5, 6, 24, and 48 h postdose, was analyzed for glucose by a previously described method (21). The maximum percent rise in blood glucose and any permanent glycosuria were the test observations made on the candidate agents.

The two dialuric acid derivatives, VIa and b, were inactive at 50 mg/kg ip; VIc was evaluated at 100 mg/kg and was similarly inactive. The thiobarbiturate IVb was inactive at 43 mg/kg, and the iminobarbiturate Vd was inactive at 50 mg/kg. However, the 5-(2,4-dichlorophenyl)iminobarbituric acid (Va) was toxic to all three rats at 100 mg/kg and produced a marked, but transient, rise (*i.e.*, 48% at 2 h) in blood glucose with no permanent hyperglycemia and no toxic deaths at 25 mg/kg ip.

The doses employed in this study were below the diabetogenic levels usually reported for alloxan-induced diabetes in rats (22), but they were in vast excess of the levels which would be employed in radiolabeled, carrier-free, radiopharmaceuticals. The absence of any permanent glycosuria at 48 h postdose and the transient rise in blood glucose (for Va) indicate that at these concentration levels, the alloxan derivatives are probably not cytotoxic to sufficient β-cells to be diabetogenic. The one active imine (Va) may, in fact, induce hyperglycemia by an *in vivo* hy-

drolytic scission to alloxan itself; electron-withdrawing halogens on the aryl ring would be anticipated to activate the anil to hydrolysis. Compound Va would appear to be an attractive candidate for radiohalogenation and study as an insulinoma-imaging agent with the caveat that *in vivo* hydrolysis may separate the label-bearing aniline and thwart target uptake of the tracer.

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Analysis of Gossypol and Gossypol–Acetic Acid by High-Performance Liquid Chromatography

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Received September 16, 1982, from the *Department of Pharmacognosy and Pharmacology, College of Pharmacy, University of Illinois at the Medical Center, Chicago, IL 60612.* Accepted for publication January 10, 1983. *Present Address: Faculty of Pharmacy, Cairo University, Cairo, Egypt.

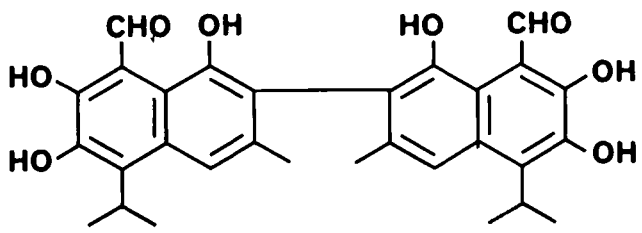
Abstract □ Gossypol, a bis-sesquiterpenoid cotton pigment, is of current interest as a male fertility-regulating agent. For the purposes of analyzing material to be studied biologically, a method is described for the analysis of gossypol by high-performance liquid chromatography. This has been used for examining the purity of gossypol–acetic acid using a UV-absorbance ratio technique.

Keyphrases □ Gossypol—analysis, gossypol–acetic acid, high-performance liquid chromatography □ Contraceptives, male—gossypol–acetic acid, analysis, high-performance liquid chromatography

Gossypol (I), one of several pigments isolated from *Gossypium* (Malvaceae) (1, 2), is found in concentrations of up to 1.7% in cotton seeds (*G. hirsutum* L.). Because of the toxicity of gossypol, cotton seed flour has limited use for humans and domestic animals; the upper limit of gossypol concentration for human consumption has been set at 0.045% (3). Gossypol is reported to be unstable and is, therefore, usually available as the 1:1 complex with acetic acid (1, 2, 4, 5)[1].

Several analyses of gossypol have been described, including complexation with aromatic amines followed by UV analysis (6, 7), GC analysis of the trimethylsilyl ether (8) and *N,O*-bis(trimethylsilyl) acetamide derivatives (9), paper chromatography (10), and TLC (11). A recent (12) communication describing the high-performance liquid chromatographic (HPLC) analysis of gossypol prompts us to report our own efforts in this area[2].

Our interest in gossypol was stimulated by reports that it possessed *in vitro* spermicidal activity (13, 14) and *in*



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[1] For an extensive review of early chemical work on gossypol see Ref. 1; for a broader discussion of gossypol see Ref. 2.

[2] This work was first presented at a meeting of the Core Group of Advisors to the Chemical Synthesis Programme, Task Force on Long-Acting Agents for the Regulation of Fertility, World Health Organization, held in Bethesda, Md., November 1980.

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