Table 1

Species	Origin	Extractive identified
Carapa guayanensis	Brazil[6]	Andirobin and 7-deacetoxy-7-oxogedunin
C. procera	Southern Nigeria	Carapins *A* and *B*
Cedrela odorata	,, ,,	7-Deacctoxy-7-oxogedunin and *Cedrela odorata* substance *B*
C. odorata	Jamaica[5b]	Methyl angolensate
Ekebergia senegalensis	Northern Nigeria (native)	8-Methoxy-4-methylcoumarin and ekebergolactones *B* and *C*
E. senegalensis	Southern Nigeria (cultivated)	Ekebergolactones *B* and *C*
Entandrophragma angolense	Southern Nigeria	Gedunin and/or methyl angolensate
E. candollei	,, ,,	—
E. cylindricum	,, ,,	Entandrophragmin
E. utile	,, ,,	Entandrophragmin and utilin
Guarea cedrata	,, ,,	—
G. thompsonii	Southern Nigeria[10]	Dihydrogedunin and/or methyl angolensate
Khaya anthotheca	Southern Nigeria	Anthothecol
K. grandifoliola	,, ,,	Khivorin
K. grandifoliola	,, ,,	Methyl angolensate and *Cedrela odorata* substance *B*
K. ivorensis	,, ,,	Khivorin
K. senegalensis	,, ,,	7-Deacetoxy-7-oxokhivorin
K. senegalensis	Northern Nigeria	Complex mixture
Lovoa trichilioides	Southern Nigeria	—
Pseudocedrela	,, ,,	7-Deacetoxy-7-oxogedunin
Trichilia heudelotii	,, ,,	—
T. prieuriana	,, ,,	Prieurianin
Turreanthus africana	Ghana	Turraeanthin

in the usual manner. As solvent, either benzene : ethyl acetate (3 : 1) or isopropyl ether : methanol (11 : 1) was used. All extracts were chromatographed in both solvent systems with gedunin as standard reference substance. To detect the spots, the chromatoplates were heated at 200° for 10 min, and sprayed while hot with a saturated solution of antimony trichloride in chloroform. All extracts gave a single violet spot corresponding in colour and R_F value to β-sitosterol. Other plates were sprayed with an aqueous solution of ferric chloride, followed immediately by 0·1 M sodium molybdate. This showed up the meliacins present as white spots on a yellowish background; there were usually one to four spots obtained from each sample in this way. These were identified by comparing their R_{Ge} ($R_{Ge} = R_F$ substance/R_F gedunin) values with those of pure substances isolated from large-scale extractions of timber.

Using this technique, we have examined several hundred samples of wood from twenty species of the Meliaceae. In every case where the timber was subsequently examined on a large scale, the constituents isolated agreed with those identified chromatographically. This has been particularly useful in dealing with variable species, for example, in selecting timber to extract for methyl angolensate which occurs in about 10 per cent of Nigerian *Entandrophragma angolense*. As most species give a characteristic pattern of extractives, this method can also be used as a means of identifying timber samples: for example, *Khaya anthotheca* is readily distinguished from other *Khaya* species chemically, while this is not easily done by the usual methods of timber identification.

Specimen chromatograms of the species examined are shown in Figs. 1 and 2. All these are of specimens authenticated by herbarium material held at the Forest Herbarium, Ibadan.

We thank Mr. J. F. Redhead and the Federal, Regional and University of Ibadan Departments of Forestry for supplies of timber, and Mr. G. Adesida for assistance in extraction of the woods and running of the chromatograms.

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PHOTOELECTRIC EFFECTS IN HUMAN BONE

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SOME evidence has already been presented indicating that the growth response of living bone to mechanical stress[1] is based on semiconducting properties of the bone matrix itself[2–4]. The basic functional unit of the matrix appears to be a *PN* junction diode formed by the precise molecular association between the collagen fibres (*N* type material) and the mineral apatite crystals (*P* type material). In has been possible to obtain approximate measures of the rectification properties of this diode including the forward and reverse current-voltage relationships and the reverse bias breakdown range[3]. It is possible, but unlikely, that the measured rectification effects were related to the anisotrophic nature of the bone matrix. Therefore, it was considered desirable to use some other method, not related to these properties, to verify the existence of the diode.

Certain specific electro-optical properties are known to be associated with manufactured mono-crystalline diodes; for example, photoconductivity with enhancement in reverse bias[5], photovoltaic effects related to the junctional barrier[6], wave-length-specific action spectra for both photovoltaic and photoconductivity effects[7] and recombination radiation at high current levels of forward bias[8]. The postulated bone diode is quite unlike these manufactured devices, being composed of two highly dissimilar materials (a macro-molecular fibrous protein and extremely small crystals of a mineral, probably apatite). It was therefore considered unlikely that exactly equivalent properties would be demonstrated by it. Nevertheless, the identification of some similarities would lend support to the bone diode hypothesis and possibly furnish additional information on the nature of this unusual structure.

Specimen preparation. Bone samples were obtained from fresh lower-extremity amputation specimens. All samples represented clinically normal bone, the amputation being necessitated by mechanical or traumatic considerations. The per osteum, a thin layer of subperiosteal bone, the marrow and all cancellous bone were removed and the remaining dense cortical bone split into 5 × 10 × 20 mm sections. These sections were exposod to normal room temperature and humidity for 10 or more

days, and were then reduced to 2 × 5 × 6 mm size by hand sawing and polishing on a series of abrasive papers. The largest dimension represented the original long axis of the bone. The final specimen was considered to consist primarily of the collagen fibre-apatite crystal matrix with a full complement of structured water and a variable amount of free water (dependent on room humidity level). The original cellular content of this cortical bone is reported to be less than 5 per cent[9] and after this preparation was considered to represent a residue unimportant to the study at hand. Samples of bone apatite alone were prepared by refluxing whole bone samples, of the same size, with ethylene diamine[10]. Removal of the apatite crystals (demineralization) was done by prolonged agitation in 5 per cent formic acid, a method reported to have little or no deleterious effects on the collagen fibres[11].

Electrodes and test fixtures. Colloidal silver paint was used for the primary electrodes. This material adhered well to the clean bone surface and provided good electrical contact. 'Aquadog', indium-mercury and various other amalgams were tried but provided unsatisfactory contact. After application of the silver paint electrodes, the bone sample was placed in a solid polyethylene fixture permitting a pressure contact between 30-gauge spring platinum wires and the primary electrodes. A second coat of silver paint was applied to ensure good electrical contact between the wires and the deposited silver electrodes. The test fixture with attached sample, biasing supplies (when used) and the first stage electrometer tubes, were placed in a solid-walled aluminium box with a controllable diaphragm permitting sample irradiation. The specimen box and all other equipment were placed within a double-walled Faraday cage, the box being earthed to the inner shell of the cage.

Electrode placement. Bone is a highly organized material from the gross macroscopic to the molecular level. At the former level, bone has an overall linear arrangement with collagen fibres lying parallel to each other and effectively in line with the original long axis of the bone. At the molecular level, the apatite crystals—some 500 Å in long-axis dimension—are applied to the surface of the collagen fibres in precise relationship to the 640 Å repeating band structure of the fibres[12,13]. Interstices between fibres are filled with additional apatite crystals. Because of this structure, it was postulated that an electrode applied to a cross-sectional cut surface would primarily contact collagen fibres, while one applied to a longitudinal cut surface would contact primarily apatite crystals. This arrangement of electrodes would theoretically give us access to a population of diodes essentially in parallel (because of the extremely small size of the individual units it is obviously impossible to make direct contact with any single one). Admittedly, this method has many defects, not the least of which is the considerable element of chance in procuring an actual diode contact and not merely a resistive contact with each electrode 'seeing' equal numbers of collagen fibres and apatite crystals. However, the method was previously used, and rectification properties were found in a number of samples, according with the general thesis[3]. All experiments reported in the present study utilized this 'diode' electrode placement except those concerning surface photoconductivity. In this case, 'surface' electrode placement, that is, both electrodes on the same longitudinal cut surface, was used. In all cases, direct illumination of the electrodes was avoided, so far as possible, by using a small focused spot of light (2 mm diameter) and by shielding the electrodes by opaque masks.

Apparatus and techniques. The photocurrents generated were measured with an electrometer amplifier (Kiethley 603) with resistances of from 10^{14} to 10^{11} ohms. The output was recorded on a dual channel recorder, the second channel being used to record the light signal. A 100-watt zirconium arc was used as the primary white light source with appropriate lenses to produce the focused spot. The light source, lenses and specimen box were mounted on an optical bench to maintain alignment. Action spectra determinations in the visible range were made with a Bausch and Lomb grating monochromator and tungsten source. This apparatus was usable to 7500 Å with precision. The range from 7000 to 20,000 Å was covered by a series of Corning glass filters with a quartz iodine lamp as the radiation source. Responses to each filter were corrected for percentage filter transmission and energy radiated by the source in the spectral range of the filter. Biasing voltages for photoconductivity measurements were obtained from mercury cells and normally ranged from 50 mV to 1,350 mV. In all experiments illumination was maintained until maximum responses had been obtained.

Since initial experiments indicated that polarization phenomena would be encountered, single determinations were made and the samples 'shorted out' between individual experiments. A stable base-line was always obtained before a determination.

Photoconductivity effect. Specimens prepared with surface electrode placement demonstrated definite photoconductivity phenomena throughout the range of biasing voltages. Rise times were rapid, averaging 2–6 sec to maximum response. However, this current immediately began to decline, and reached the original base-line in 10–30 sec. If the illumination was stopped at this point an exactly similar pulse, but with opposite polarity, was obtained (Fig. 1). These results were interpreted as indicating a charge storage or polarization type of phenomenon[14]. Changing the direction of the applied field had no effect on the amplitude or type of photocurrent obtained.

The diode electrode configuration also demonstrated photoconductivity phenomena but differed sufficiently from the surface electrode configuration to indicate that true 'bulk' photocurrents were being measured. Rise times were considerably longer, with a wide range of values, averaging 200 sec. Peak responses were maintained without decrement as long as illumination was continued and the photocurrent fell to the original base-line after the light was turned off without demonstrating a pulse of opposite polarity. Within the range of biasing voltages used, reverse bias (RB-positive pole of battery to the collagen electrode, negative to the apatite electrode) was invariably productive of a greater photoconductivity response than forward bias (FB) (Fig. 2). When electrode placement failed to make a true 'diode' contact, not only did the specimen not demonstrate rectification properties but the photoconductivity phenomena were absent, or low in amplitude, and did not vary with change in bias direction. With any single determination, in either forward or reverse bias, there was no evidence of polarization as was seen with the surface electrode placement.

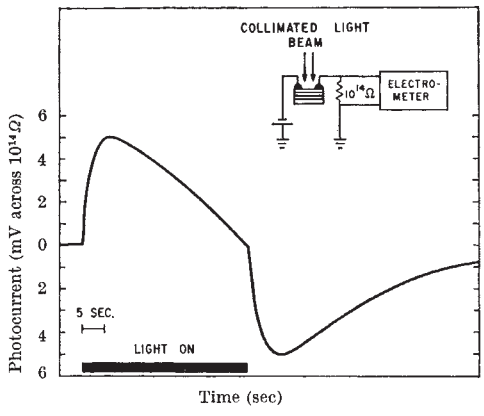

Fig. 1. Surface photoconductivity in whole human bone. The experiment is set up as shown (upper right). The 'grain' of the bone sample is indicated by the parallel lines

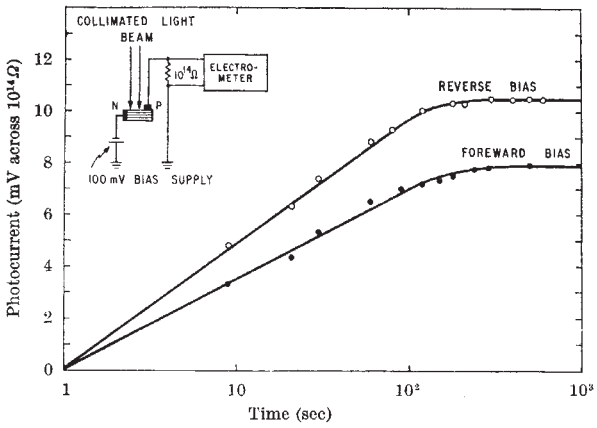

Fig. 2. Bulk photoconductivity in whole human bone. The path of the current flow between the electrodes is across a population of collagen-apatite junctions. The rise time of the response is approximately 40 times longer than that of the surface configuration and is shown on a semi-log scale

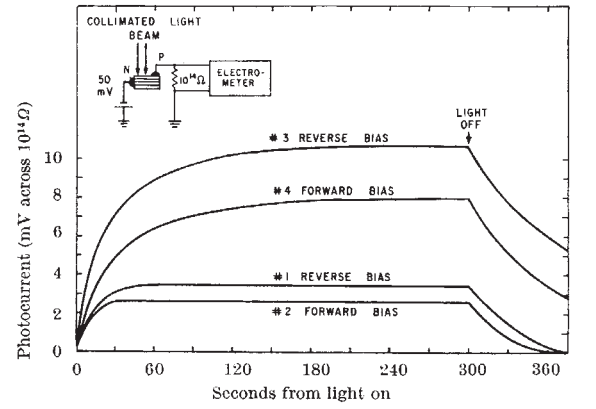

Fig. 3. Bulk photoconductivity for several sequential runs of forward and reverse bias. The sequence of the experimental runs is indicated by the number over the centre of each response curve. The photocurrents are charted on a linear scale in this illustration and show three major differences with the surface photocurrents: prolonged rise time, steady continuous response to illumination and exponential fall to base-line when light turned off. Note that in all cases the reverse bias response was greater than the forward bias. The same bias voltage was used throughout

However, a second series of forward and reverse bias experiments was significantly higher in amplitude (still maintaining the RB > FB relationship) than the preceding ones at the same bias voltage (Fig. 3). This phenomenon was observed to hold for up to three cycles, after which responses became aberrant. If this represents internal polarization, the field generated is such as to increase rather than decrease the response.

Photovoltaic effect. Specimens that previously demonstrated photoconductivity with responses in reverse bias > forward bias were used to search for photovoltaic effect. Definite effects were noted with characteristics (rise time, magnitude of response, sustained response, etc.) similar to those previously noted in forward biased conditions. Cooling the specimens to 77° K with liquid nitrogen resulted in approximately 40-fold increases in the magnitude of the response, but with some prolongation in the rise time (Fig. 4).

Action spectrum. The action spectra obtained were similar in both photovoltaic and photoconductivity conditions. Whole bone consistently demonstrated three major spectral peaks; 4100 Å, 4800 Å in the visible range and a broad response between 8500 and 10,500 Å with a peak approximately at 9500 Å in the near infra-red (Fig. 5). A minor peak was noted inconsistently in the vicinity of 5700 Å. The characteristics of the photocurrents generated were similar in each spectral area and frequently the 9500 Å range demonstrated a rise time faster than that in the visible ranges. Therefore, this response to near infra-red is considered to be a true photoelectric effect and not merely the result of heating the sample. Liquid nitrogen temperatures increased the amplitude of the response approximately ten-fold in the two visible ranges and 50-fold in the infra-red region. Samples of highly purified bone apatite demonstrated a single peak in the 4800 Å region. Demineralized samples, however, demonstrated spectra quite similar to that for whole bone except that the responses in the 4800 Å and 9500 Å regions were diminished and the 5700 Å response was frequently missing. It appears probable that the formic acid demineralization technique, while producing a flexible specimen quite suitable for microtome sectioning, does not completely remove the apatite crystals functionally bound to the collagen fibres. Spectrographic analyses of similar demineralized bone samples demonstrated considerable quantities of calcium still present (see following article). It is suggested that this method of demineralization primarily removes the interstitial apatite crystals.

Influence of water content on action spectrum. It is known that mature cortical bone contains a small, but significant, amount of water *in vivo*[9]. Presumably this is bound or structured water associated with the collagen fibres. In the course of this work, examination of the bone samples, which were exposed to room humidity, revealed what appeared to be a thin film of free water on their surface. It was postulated that our samples acquired excess water through some hygroscopic action. The presence of such free water would presumably tend to

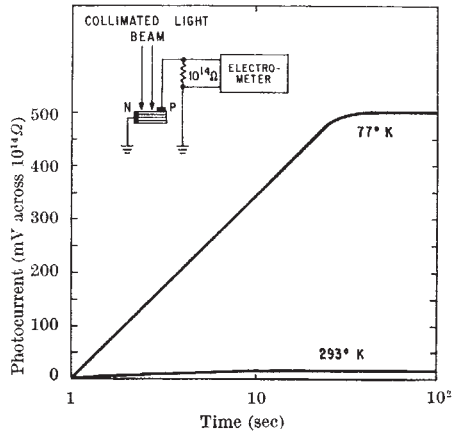

Fig. 4. Photovoltaic effect in whole human bone

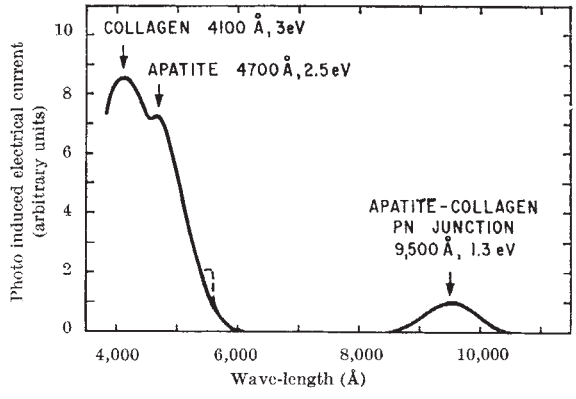

Fig. 5. Photoconductivity action spectrum of whole human bone. The exact configuration of the response band centred at 9500 Å is not known since this section of the spectrum was investigated with filters having half-power band-pass limits of 250 Å on the average. However, the peak response can be centred at 9500 Å with fair certainty

decrease the photoelectric responses, and it was considered desirable to evaluate this condition.

Since heating beyond 100° C would produce irreversible damage to the collagen fibres, vacuum sublimation techniques were utilized to reduce the water content of whole bone samples. The specimens were placed in 'Vycor' (Corning) tubes with one flat side optically finished. Initial action spectra determinations were made with these samples equilibrated to room humidity. The tubes were then placed in a vacuum chamber and pumped down to less than 0·1″ of mercury and repeatedly flushed with dry nitrogen. They were maintained at this pressure for 72 h, after which dry nitrogen was again admitted to the chamber and the tubes were quickly sealed. Action spectra were immediately determined revealing consistently a complete absence of the 4100 Å peak, slight but inconsistent variations in the 4800 Å range, and a slight increase in the magnitude of the 9500 Å response. The seals were then broken, permitting the samples to re-equilibrate to room humidity. Action spectra were determined at intervals during this process. The 4100 Å peak reappeared and increased to about twice its original amplitude in 2–3 h. The 4800 Å peak increased about three-fold at the same time, while the response in the 9500 Å range increased about five-fold. These values then gradually declined until after 4–5 h the initial values for each range were reached.

Conclusions. Human cortical bone, prepared as in this study, demonstrates definite photoconductivity and photovoltaic phenomena, unassociated with surface states or the electrode-bone interface. The enhancement of photoconductivity responses by reverse bias as compared with forward bias, and the demonstration of temperature dependence of the photovoltaic effect, indicate that the material has diode properties in accord with the theory previously developed. The action spectra with responses at several wave-lengths is considerably different from that of the monocrystalline diodes. However, since the hypothetical diode was formed by the association between two very dissimilar semiconductors, a three-peaked spectrum was expected. It was expected that responses to two different wave-lengths would be produced by the two materials, while a third response, resulting from the diode zone itself, was predicted. The equivalent energy gaps for the wave-length-response areas associated with the two components should be close to those determined by other methods. The observed spectra conformed surprisingly closely to these theoretical criteria. The 4100 Å response is considered to be associated with the collagen fibres since it was absent in the decollagenated samples, and disappeared in the completely dehydrated whole bone samples. The equivalent energy, 3·0 eV, is quite close to the values derived for polypeptides and other proteins[15–17] but different from that previously obtained for bone collagen (5·0 eV) by thermal effects on d.c. conductivity[3]. However, the present study indicates that the samples of bone collagen used in that study were not as pure as had been thought and the figure of 5 eV is considered to be in error. The 4800 Å peak appears to be unquestionably related to the apatite mineral indicating a band gap of approximately 2·5 eV. The 9500 Å area is considered to represent the diode zone proper with an energy gap centred at 1·3 eV since it is present only in samples having both components and is markedly enhanced by liquid nitrogen temperatures. The minor inconsistent response at 5700 Å is presently considered to be associated with one of the soluble organic components of the matrix.

The hydration experiments appear to indicate that bound water is an important factor in collagen conductivity, in agreement with determinations on other proteins[18]. However, bound water seems to be of much less functional importance for the apatite and for the diode zone itself. The high values for all components in the partially rehydrated specimens are interpreted as representing re-acquisition of the complete bound water components by the apatite–collagen complex. The subsequent decline in magnitude with continuing re-equilibration to normal room humidity is perhaps the result of hygroscopic action producing a free-water compartment providing shunting resistance to the diodes.

Thus, despite the marked difference in structure between the theoretical apatite–collagen diode and inorganic monocrystalline diodes, surprising similarities are apparent in their photoelectric behaviour. The results appear to support the thesis that the apatite–collagen complex has the properties of a semiconducting *PN* junction diode.

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FLUORESCENCE OF BONE

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An earlier paper[1] reported the semiconduction characteristics of bone and described its piezo-electric properties as related to the selective growth of bone in response to stress. Results of our further studies are incorporated in this report and in the preceding article. Here we describe the visible fluorescence of bone under excitation by ultra-violet radiation of 3650 Å wave-length. Although there is casual mention in the literature of the fluorescence of teeth, shells, and other calcium-rich materials we have been unable to find any reference to the fluorescence of bone.

Bone consists of a three-dimensional matrix of two basic components, the mineral apatite and the protein collagen, and a certain amount of 'ground substance'[2]. There is a